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Optimising leucaena-based forage productivity

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Abstract

Leucaena is the most persistent, productive, sustainable and profitable legume option for clay soils in central Queensland, and increasingly, for southern and northern Queensland. Leucaena-grass pastures are normally planted with leucaena as hedgerows with tropical grasses in the inter-row.

However, recommendations for between hedgerow spacing and within hedgerow leucaena density are not consistent and not based on evidence of the effect of planting configuration on pasture productivity, and most importantly on the balance of legume and grass for grazing animals.

This study has addressed this gap. A range of leucaena densities and associated grass was established in the field, on research stations and in controlled environment at the University of Queensland.

It was concluded, that leucaena planting density and especially hedgerow spacing plays a key role in determining the percentage of legume and grass in the pasture, and therefore in animal diet. For leucaena, productivity is determined by below-ground competition with the grass for plant available water; and for grass, productivity is determined by above-ground competition for light. This assumes no nutrient deficiencies.

Accordingly, wide hedgerows of leucaena greater than 6-8m will lead to dominance of the grass to the detriment of leucaena contribution to the pasture. Narrow rows less than 6-8m will lead to dominance of leucaena to the detriment of grass contribution to the pasture and grazing animals.

Executive summary

Introduction

Leucaena continues to be the most persistent and highly productive legume option for pastures on clay soils throughout central Queensland and, increasingly, for areas of southern and northern Queensland. It is relatively expensive to establish and returns are sensitive to establishment costs and productivity gains. Leucaena hedgerows provide the high nutritional quality component of pastures but inter-row grass drives overall carrying capacity. Hedgerow row spacing has a marked impact on the absolute, and relative yields of leucaena and grass, influenced by competition for moisture and sunlight, moderated by input of legume nitrogen into the soil.

Recommendations for within hedgerow density and between-row spacing for leucaena-based pastures are inconsistent and not based on solid evidence of the impacts on establishment outcomes, water use efficiency, or pasture productivity. The proposed research will address this deficiency through field work in which a range of leucaena densities and associated grass were investigated in the field, in controlled environment, and on the University of Queensland Research farm at Gatton in southeast Queensland.

The objectives of the project were as follows:

1. Measure root architecture and soil water extraction patterns of the leucaena and grass components of pasture, including the impact of within-hedgerow leucaena plant density;
2. Determine the impact of leucaena hedgerow spacing, and associated competitive interactions, on partitioning of total water use between leucaena and grass;
3. Determine the ability of leucaena and grass to extract soil water from different depths in the soil profile;
4. Quantify seasonal changes in leucaena and grass evapotranspiration crop factors;
5. Evaluate the impact of degree of defoliation of leucaena and inter-row grass on rates of recovery of plant water use and forage production; and
6. Provide management recommendations for hedgerow system design (including row spacing and row density) and grazing management that will reduce establishment costs and optimise forage productivity for varying levels of soil water availability in representative agro climatic environments.

Methods – How the work was done

Several experiments were conducted to address these objectives. Firstly, detailed monitoring of a commercial leucaena-grass pasture system was carried out in southern inland Queensland. The aim was to identify the significance of below-ground competition and level of complementarity between tree and grass components. Secondly, a series of experiments were conducted in a controlled glasshouse environment at the University of Queensland, St. Lucia campus, Brisbane; and thirdly, three field experiments were carried out at the University of Queensland farm on Gatton campus.

Results - What we found.

Monitoring experiment. The analysis of the very detailed monitoring data from the commercial leucaena-grass pasture, which used high-technology soil moisture sensors, provided water use data for two years and surprisingly highlighted that there was minimal spatial and temporal complementarity of water use between the tree and grass components,

We expected that the leucaena trees would acquire water from different soil strata when grown in association with grass; however, a high level of competition for water occurred in the top 1.5 m of the soil profile. Analysis of the data from both years showed marked seasonal fluctuation in water extraction, with

deepest and greatest soil water uptake occurring during the first and wetter growing season; while soil water extraction was greatly reduced during the cooler and drier second season. This was related to the negative influence of lower soil moisture content, lower temperature and greater defoliation on overall pasture growth. Another significant outcome of the analysis was the low amount of deep drainage measured below 4 m depth. Nevertheless, unsurprisingly, highest values of deep drainage occurred when high rainfall events corresponded with high soil water storage in the top 3 m of the soil profile.

Field trial at Gatton. The effect of leucaena plant density on above- and below-ground competition with grass was investigated using a Nelder fan design established at Gatton research station. Ten leucaena densities, from 100 to 80,000 trees/ha, growing with and without Rhodes grass competition, confirmed that density of leucaena had a strong effect on yield of leucaena and grass. Contrary to expectation, the combination of leucaena and grass did not improve the overall above-ground yield of the pasture due to strong competition for light and water. Strong grass competition reduced leucaena yield by 50–70% when it was grown at low density (100 to 4,100 trees/ha) due to the grass having more dense root systems that captured more of the available soil water. Conversely, at high leucaena density, there was minimal grass competition due to shading by the taller leucaena component.

There was strong overlap in the root distribution of leucaena and Rhodes grass and thus a low degree of complementarity in their use of water resources as root abundance of both species was concentrated in the top 1.5 m of the soil profile. At low leucaena densities, leucaena-grass pasture had higher water use efficiency (WUE) than sole leucaena, grown in absence of grass. However, this difference was reduced with increasing leucaena density due to the reduced yield of the grass component.

Water use of leucaena was successfully monitored using the sap flow measurement technique. Leucaena trees grown at low densities (100 trees/ha) had average daily transpiration rates of 23.2 litres/day/tree equivalent to 2-3kL/ha. Water uptake was reduced to 0.7 litres/day/tree at high density (80,000 trees/ha), equivalent to 56 kL/ha due to the high number of trees.

The effect of within hedgerow plant spacing on temporal and spatial water use and root patterns of leucaena was investigated under a rainout shelter facility at Gatton over a two year period. Four dry-down periods were evaluated and the last extended for one year without soil water inputs from either rainfall or irrigation. Unexpectedly, leucaena density within-row did not affect water use and rooting patterns of leucaena. Leucaena plants grown at wider within-row spacings (1 plant/m) had the plasticity to generate increased root, branch and leaf biomass and thus capture the same amount of water as when grown at closer within-row spacings (10 plants/m). Periods of water uptake without water limitation were characterised by extraction of water from the upper soil profile (0-1m). However, during the long dry period, there was evidence that leucaena had the ability to extract more water from deeper in the soil profile.

The effect of defoliation of leucaena on plant water use was investigated in a controlled glasshouse environment. In general, results indicated that increasing intensity of defoliation reduced plant water uptake. Severe defoliation by cutting to 1 m height reduced cumulative soil water extraction by 79%. Surprisingly, a light defoliation treatment (25% removal of leaf) stimulated leaf and shoot growth and increased water uptake compared to undefoliated leucaena.

What are the industry benefits arising from this work

In general, very little complementarity was observed between grass and leucaena due to the intense competition for soil water occurring in the top 1.5 meter of the soil profile.

In terms of practical recommendations, thought needs to be given as to the most appropriate leucaena hedgerow spacing and plant density which will vary according to the method of production. Plant water availability should be considered as the main determining factor in choice of hedgerow configuration. For instance, some graziers plant wider leucaena hedgerows (>10 m apart) arguing that leucaena plants will have better access to soil moisture and will perform better in dryland areas. However, this project has demonstrated that this practice will promote grass growth at the expense of leucaena production. Therefore, in drier regions with rainfall <800mm, closer rows (6-8m) should be planted to reduce grass competition effects and increase the availability of water for the higher quality leucaena component. In addition, this study has provided clear evidence that leucaena is adapted to dry conditions, and patterns of water uptake and rooting distribution can change according to plant available water in the soil profile. For instance, during dry times, leucaena does increase the amount of water extracted from deeper in the soil profile. Perhaps, in areas of high rainfall, or under irrigation, wider row spacing <6-8m can be recommended to increase the amount of inter-row grass available without reducing leucaena growth within the hedgerows. This would have the advantage of permitting higher stocking rates, and better height management of leucaena hedgerows which are prone to excessive growth in high moisture environments.

Key additional messages for industry include:

- Leucaena can extract soil water to 3 m depth and deeper in dry times demonstrating its drought tolerance. Very little deep drainage of soil water occurred below the leucaena root zone and only following large rainfall events in those rare circumstances when the profile was already full to 4m. Frequent large rainfall events (i.e. above average rainfall seasons) would be required to replenish deep subsoil moisture beyond the grass root zone to selectively promote leucaena growth.
- The work has clearly demonstrated that grass competition should be eliminated during leucaena establishment as grass competition for soil moisture reduces above-ground growth and significantly reduces leucaena rooting exploration, water uptake and biomass yield. The importance of fallowing paddocks to store a profile of soil moisture prior to leucaena establishment and then to maintain complete weed and grass control until leucaena is over 2m tall is emphasised
- Increasing the leucaena population in the pasture by using narrower row spacings, will reduce grass competition for soil water and promote higher yields of leucaena component of the pasture.
- Within hedgerow populations of the leucaena plants at densities of one or 10 plants per metre of row, did not affect leucaena rooting pattern or extent of soil water extraction. Further study of even lower within-hedgerow density is required to determine the critical plant density below which yield is compromised.
- Leucaena defoliation management can affect water use, reducing almost to nil under severe defoliation, while mild pruning (<25%) can stimulate water uptake of leucaena.

Future work

The Nelder fan experimental site should be maintained and monitored in the ensuing years as interactions between trees and grasses will change with age.

It would be valuable to study the effect of plant density on soil carbon and nitrogen fixation using the Nelder fan experiment.

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1 Background

1.1 Leucaena-grass pasture in Queensland

Integrating trees, grasses and livestock has the potential to diversify income, reduce financial risk, lift profit and enhance environmental benefits (Murgeitio et al. 2011; Cabbage et al. 2013) in both tropical and temperate regions (Carvalho et al. 2001; Nair et al. 2004; Montagnini et al. 2015). In silvopastoral systems, forage tree legumes have been used to achieve this goal.

Leucaena [*Leucaena leucocephala* Lam de Wit. ssp. *glabrata* (Rose) Zarate] is the principal multipurpose forage tree legume widely used for ruminant feeding in subtropical and tropical regions (Shelton and Brewbaker, 1994). In Queensland Australia, leucaena-grass pasture is the most productive, profitable and sustainable pasture option for cattle production (Shelton and Dalzell, 2007) due to its superior nutritive characteristics compared to pure stands of grass e.g. Rhodes grass (*Chloris gayana* Kunth.) (Dalzell et al. 2006). Ruminants grazing leucaena also have 23% reduced greenhouse gas emissions (Harrison et al. 2015). More than 200,000 ha of leucaena-grass plantation has been established in Queensland, Australia (H. M. Shelton, personal communication).

Surprisingly, little is known about the ecological interaction between trees and grasses when these differing species are grown together (Ong et al. 1991). When the interaction is negative it becomes competitive; when the interaction is positive, complementarity between the species can be expected (Jose et al. 2004). Competition for light is the common limitation when water and nutrients are available as the tree component is taller than understory grasses and thus has an advantage in light capture. However, in the early phase of tree establishment, the grass can strongly compete for light as well as water resources due to its more rapid development of canopy and root system which can cause tree seedling mortality. Water is an especially limiting factor in semi-arid regions and nutrients can be limiting in acid, leached or degraded soils (Ong et al. 1991).

The interactions between trees and grasses are generally classified as above- and below-ground (Singh et al. 1989; Ong et al. 1991). The common above-ground interactions are microclimatic modification and competition for light. Below-ground interactions occur when trees and grasses explore the same soil strata and compete for water and nutrients. Although there have been significant advances in agroforestry research during the last decades, focusing on the interaction between components (tree, grass, crop, animal), there is a greater understanding of above-ground interactions than below-ground interactions and how tree density can affect these interactions.

In leucaena-grass pasture there is limited understanding of the above- and below-ground interactions that occur between trees and grass. This is a major obstacle to enhanced design and management of these systems; these interactions will now be reviewed.

1.2 Above- and below-ground interactions

Above-ground interactions

In tree-grass combinations, the shade of the trees can modify microclimate factors such as soil temperature, soil evaporation, wind speed and light available for grasses. This will affect the quantity and quality of the forage produced. The response to shading will depend on the differences in carbon fixation pathway at the species, where C3 plants are more adaptable to shade than plants with the C4 pathway (Lambert, 1998). It is

well known that the photosynthesis rate at C3 plants increases as photosynthetically active radiation (PAR) increases from deep shade up to 25-50% full sunlight, remaining light-saturated as light increases further. Meanwhile, C4 plants do not become light-saturated and photosynthetic rate increases up to full sunlight. Tree canopies can be modified by pruning, thinning and planting configuration with the aim to provide a better light environment for understory grass. The tree canopies can also reduce extreme temperatures compared to an open pasture. This can have a positive effect reducing heat stress on crops, grasses and animals in agroforestry systems.

Below-ground interactions

A fundamental hypothesis of agroforestry is that root systems of trees and pastures occupy, to some extent, different soil strata when grown in association, leading to a degree of complementarity in their use of soil resources (Schroth, 1999). It is argued that the trees can acquire resources that the crops or pastures would not otherwise acquire (Cannell et al. 1996). However, when trees and grasses are overlapping in the root zone competition for water and nutrients occurs. In this situation, it is difficult to separate interactions for water and nutrients, although it is recognized that in semi-arid regions the main competition will be for water (Ong et al. 1991; Jose et al. 2004). Deeper and denser rooting systems in multi-species systems may exploit the soil more completely, increasing the potential for water and nutrient uptake (Ong et al. 2004). Trees with dense mats of shallow roots are likely to compete more with crops and pasture for water and nutrients than trees with deep roots (Akinnifesi et al. 2004).

The rooting depth of tree and grass species growing in combination is an important factor to consider in agroforestry. Although, the root distribution depends on many factors such as species, genotype, chemical and physical soil properties, nutrients status and plant vigour (Akinnifesi et al. 2004); without limitation, tree roots may penetrate to great depth. Stone and Kalisz (1991) did an exhaustive root depth study of 49 families, 96 genera and 211 species and they reported that *Juniperus monosperma* roots at depths of 61 m or more in mines; other extraordinary depths reported were for *Eucalyptus* sp. (60 m), *E. calophylla* (45 m), *E. marginata* (40 m), *Prosopis juliflora* (over 53 m) and *Acacia raddiana* (35 m). With these data, they demonstrated that roots may play a more important role in uptake of water and nutrients than indicated by their density alone. Only a few agroforestry tree species with deep roots have been reported. It could be that studies of agroforestry systems rarely sample rooting depth beyond 2 m as they tend to focus on the rooting of the crop (Akinnifesi et al. 2004). In Australia, the deepest roots of *L. leucocephala* were reported by Poole (2003) where roots were found to a depth of 6 m under leucaena (5-10 years old). This was a similar rooting depth to that of the native forest species. Other studies have reported presence of roots at 2.8 m in 28 month old leucaena (Dhyani et al. 1990), 2.6 m in 38 year-old leucaena in alley cropping with pasture (Radrizzani, 2009) or 2 m in an alley cropping system with maize (Rao et al. 1993). Both of these studies had a restrictive rock layer to this depth.

Root distribution will vary according to plant species and physical and chemical soil condition. A global analysis of root distributions from a database of 250 root studies was conducted by Jackson et al. (1996). They concluded that the average global root profile was approximately 30% of roots in the top 0.1 m, 50% in the top 0.2 m and 75% in the top of 0.4 m. When they compared rooting patterns for various plant functional groups such as grasses, shrubs and trees, they found that grasses had 44% of their root biomass on average in the top 0.1 m of soil, whereas shrubs had only 21% of their roots to the same depth. In addition, grasses had 75% of their root biomass in the top 0.3 m compared to 47% for shrubs. According to Schroth (1999), "it is important to distinguish between the total depth explored by a root system and the distribution of the roots in the soil profile". Plants with shallow roots that present a rapid decline in root

mass, length and density with the increasing depth seem to be more competitive for resources than those which exhibit a large number of fine roots in the topsoil and have a substantial proportion of roots in deeper soil layers.

Leucaena plants have deep root systems that exploit subsoil moisture and nutrients beyond the reach of grass roots and this has been observed in the field (H.M. Shelton personal communication). In agroforestry studies, the distribution of fine root mass of 2 year old leucaena was similar to that of maize in the 0-1 m profile (Rao et al. 2004). Generally root length density (RLD) decreased with increasing soil depth. In a study by Normaniza et al. (2008), after 6 months all plants showed a high proportion of RLD in the top 0.8 m of soil depth (53% of the total RLD) ; after 12 months, the highest RLD was observed at 0.4 – 0.80 m soil depth with the total RLD twice that of plants at 6 months.

However, the maximum root density distribution is quite variable probably due to variation in soil condition; some authors reported that most roots occur in the top 0.15 m layer (Akinnifesi et al. 2004) while Radrizzani (2009) found that the highest abundance of leucaena roots was from the surface horizon to 0.2 m depth. This zone contained an average of 43% of total leucaena roots. Other observations made by Toky and Bisht (1992) were that between 78 to 84 % of the total root biomass were contained from the surface to 0.3 m depth. Whereas, Dhyani and collaborators (1996) found that *L. leucocephala* had higher densities of fine roots from 0.3 to 0.6 m of depth.

Restrictions of lateral root development and the formation of vertically stratified root systems in the contact zone of competing root systems may be a mechanism by which plants avoid excessive intraspecific and interspecific root competition (Schroth, 1999). Trees with lateral roots confined to a distance of less than 1 m from the trunk are desirable for agroforestry systems (Ruhigwa et al. 1992). Lateral root spread of leucaena (28 months old) was 1.35 m (Dhyani et al. 1999), while Toky and Bisht (1992) observed 1.43 m of horizontal root movement in leucaena 6 years old. According to Govindarajan (1996), leucaena root spread in 4 year old trees was relatively low (1.5 m) in comparison to single leucaena trees (same age) with more than 2 m of spread; however root spread declined drastically beyond 2.5 m.

The morphology of roots is another characteristic to consider for a better understanding of below-ground interaction. Root morphology refers to the surface features of roots as an organ, including characteristics of the epidermis such as root hairs, root diameter, the root cap, the pattern of appearance of daughter roots, undulations of the root axis, and cortical senescence (Lynch, 1995). Generally, fast growing woody species tend to have deep and extensive root systems composed of two main components: the main structural roots and the fine roots. The first form a base for support and anchorage of the plant and represent around 90 % of root biomass. In contrast, the fine roots component (roots <2 mm diameter) consists of a long exploratory system with the main role of water and nutrient uptake (Akinnifesi et al. 2004).

Toky and Bisht (1992) observed rooting of 12 agroforestry species, nine of them indigenous trees, *Acacia catechu*, *Acacia nilotica*, *Albizia lebbeck*, *Azadirachta indica*, *Dalbergia sisso*, *Melia azedarach*, *Morus alba*, *Prosopis cineraria*, *Zizyphus rauritiana*, and three exotic species, *Eucalyptus tereticornis*, *Populus deltoides* and *Leucaena leucocephala*. All species had variable numbers of prominent lateral roots with the primary function of anchorage of the tree to soil and *L. leucocephala* showed a well-developed taproot like other species. In addition, they reported the numbers of primary, secondary and tertiary roots differed among species and *Leucaena leucocephala* had 807 (± 199) roots, of which 30 (± 5.2) were primary, 270 (± 80) were secondary and 507 (± 119) were tertiary. The average of the 12 species was 24 (± 11.3) primary roots, 217 (± 162.1) secondary and 465 (± 476) tertiary. The angles of primary and secondary roots, measured with respect to main root and primary roots respectively, varied considerably within and among species and

generally the angles were higher in primary roots than in secondary roots. In this study *L. leucocephala* showed an angle of 71.7° (± 4.8) in primary roots and 47.7° (± 5.3) in secondary roots.

In agroforestry systems, it is possible to manipulate tree root systems by removing or reducing the tree shoots as this will reduce the size and abundance of root activity in the soil (Akinnifesi et al. 2004). Different types of pruning in agroforestry included pollarding, coppicing and lopping according to the species used (Chesney, 2012). This practice affects competition between trees and crops by controlling water demands through reduction of leaf area and fine roots. Defoliation affects the functional equilibrium between above and below-ground components, and finally, alters fine root distribution within the soil profile (Rao et al. 2004). Pruning trees, also reduces competition for light and provides opportunities to enhance timber quality. However, there are few studies documenting changes in root morphology and function as a consequence of above-ground pruning (Rao et al. 2004). According to van Noordwijk and Purnomosidhi (1995), who studied 18 multi-purpose trees in Indonesia, light pruning induced a shallow root system with more fine and adventitious roots due to loss of apical control by the meristem of the main roots. Defoliation also reduced carbohydrate storage in stems and created hormonal imbalances. A survey carried out by Rao et al. (1993) of root systems of *Cassia siamea* and *L. leucocephala* regularly pruned and unpruned, showed that pruning affected rooting depth but apparently not root density. Akinnifesi et al. (1995) working with *L. leucocephala* hedgerows on an alfisol soil, found fewest roots in regularly pruned hedgerows. Regular pruning and biomass export significantly depressed fine root growth by 88% compared to unpruned hedgerows. It was recommended that pruning should not be initiated before deep tap roots have been developed, and that the trees should not be pruned too low (0.3–1 m height) (Rao et al. 2004). Severe pruning induces shallow roots with more fine and adventitious roots due to reduction of carbohydrates reserves in the stems and/or hormonal imbalance (van Noordwijk and Purnomosidhi, 1995) (Rao et al. 2004).

1.3 Water use in leucaena-grass pasture

One advantage of agroforestry systems is better use of resources or using it more efficiently, or both. Compared to sole crops, tree-grass combinations have a spatial and temporal complementarity of water uptake resulting in an enhanced use of available moisture (Ong et al. 1996). In terms of water use, the key question is, do trees-pastures-crops increase total harvestable produce by making more effective use of water? (Wallace, 1996). According to the author, the inclusion of trees may enhance water use efficiency. This review will now describe some important points relating to water movement in the soil and water used in agroforestry systems.

Evapotranspiration and water movement in agroforestry

The term of evapotranspiration refers to two separate processes whereby water is lost from the soil surface by evaporation and from the crop by transpiration (Allen *et al.* 1998). According to Novak (2011), the role of evapotranspiration in a root zone may be expressed by the water balance equation which refers to gains and losses of water in the soil root zone in a defined time (Δt) as:

$$P+I_r=I+R+(E_e+E_t)+I_d+\Delta W \quad (\text{Equation 1})$$

Where P and I_r are entry of water to systems as precipitation and irrigation, I is infiltration to the soil, R is surface of runoff, E_e and E_t are evaporation and transpiration, I_d is rate of water movement through bottom of the root zone which can be capillary rise or deep percolation, and ΔW is the soil root zone water change in a period of time. Generally all terms are expressed in mm of water over the soil surface during an interval of time (hours or days). However, modification of equation 1 can be made when species are mixed together where transpiration is split into E_{tree} and $E_{crop-pasture}$, or when changes to microclimate and below-

ground interaction between trees and crop or pasture root system cause modification of Ee, R and ΔW (Wallace, 1996).

Furthermore, the theory suggests that integration of tree and crop systems increases productive use of rainfall water reducing the non-productive losses of water because trees may arrest runoff, reduce drainage and soil evaporation by shading of bare soil (Smith *et al.* 2004). One example of this is the research into water balance and water use efficiency of different land uses reported by Narain *et al.* (1998). They found that the average of runoff over five years from cultivated fallow was 38% of seasonal rainfall which reduced to 28% with maize and, 21% with maize + leucaena, 13% under maize + eucalyptus and 4% with eucalyptus + grass. Water use of sole leucaena and eucalyptus tree plantations was significantly higher than other land uses.

Competition and complementarity of water use

As well as trees increasing water available for the crops (complementary), they also modify the water balance by consuming water; and this competition for water resources may deprive water from crop or pasture. However, it is clear that the success of the integration of trees, crops or pastures is related to minimizing the competition and maximizing the complementary. Cannell *et al.* (1996) expressed this as a central biophysical hypothesis for agroforestry. They suggested that in the case of water, benefits of trees with crops or pastures occur when trees are able to acquire water that the other crops are not able to otherwise acquire. Furthermore, this affirmation can be satisfied if there is a complementarity in water use of trees and crops. In addition, Smith *et al.* (2004) refer to cases in which water uptake by the root systems of trees and crops occurs from spatially discrete sources or at discrete times. In this example, water use is complementary and productive use of water can be improved without there being any negative impacts of competition.

The effect of competition for water resources was demonstrated by Govindarajan (1996) in an experiment in a semi-arid region of Kenya working with maize and *Senna spectabilis* and *Leucaena leucocephala* as tree components. His trial showed where seasonal rainfall was insufficient to recharge soil below the crop rooting zone, the yield of maize in alley cropping systems was reduced by between 39% and 95%. Similar results were mentioned by other authors such as Rao *et al.* (1990) and Corlett *et al.* (1992) in semi-arid tropics where severe competition between the extremely vigorous *L. leucocephala* and associated annual crops reduced crop yield by 50%-80%. However, Ong *et al.* (1991) reported that, during a continuous period of five years (1984–88), the annual cropping systems on Alfisols produced a total dry matter of 21.4 t ha⁻¹ compared to 32 t ha⁻¹ in the *L. leucocephala* agroforestry systems (4.4 m between hedges). These data suggested that annual crops could not utilize available water and the agroforestry systems may enhance productivity using a large proportion of the annual rainfall (Ong *et al.* 1996). In a study of the effect of tree density on water use by silvopastoral systems in Queensland, Eastham and Rose (1990) showed that high tree densities of *Eucalyptus grandis* affected inter and intraspecific competition by altering distribution of root growth and hence potential for exploration of soil water and nutrients. The presence of trees reduced grass pasture root length densities, potentially decreasing their ability to compete with tree roots for soil resources. They also observed that at high tree density, a larger proportion of water uptake occurred at depth profile due to the greatly depleted surface water content and deeper and denser root systems under densely planted trees. In addition, water use increased and losses due to deep drainage decreased with increasing tree density over a period of two years (1985–1986). High density (2150 trees ha⁻¹) encouraged deeper and more extensive rooting due to intraspecific competition between neighbours which inhibited extensive lateral spread roots in the surface horizons. In contrast, the trees planted at lower density and

wider spacing experienced less intraspecific competition and were able to withdraw water at greater distances from the stem where soil water contents were higher (Eastham *et al.* 1990).

Another example of competition and complementary use of water has been showed in Grevillea (*Grevillea robusta*) and cowpea (*Vigna unguiculata*) (Howard *et al.* 1996). Trees used water below the root zone of the crop, although in seasons of low rainfall, even the relatively modest uptake of 15-30% of the total of water requirement of grevillea from the rooting zone of cowpea was likely to reduce crop yield. However, limited competition can be 'a small price to pay' for a greatly increased resource capture and overall system productivity (Howard *et al.* 1996). Fernandez *et al.* (2008) determined the extent of competition-complementarity in water use between trees (*Pinus ponderosa*) and grasses (*Festuca pallescens*). They studied water resources of both species in different periods within the grown season (spring-summer-autumn). Their results suggested that existed an important degree of complementary in the use of soil water as only approximately 20% of the water use by trees came from the upper 0.2 m of soil. Another form of facilitation is 'hydraulic lift' which is the process of water movement from relatively deeper wet soil by trees to drier upper soil that then becomes available to understory vegetation (Richards and Calwell, 1987). Over the last decades, this particular movement of water has been reported in over 60 plant species worldwide (Prieto *et al.* 2011). This process can result in significant improvement in plant water and nutrient status for shrubs and surrounding plants. There is strong evidence that the redistribution of water throughout the soil profile enhances plant nutrient uptake and enhances organic matter decomposition and thus nutrient mineralization rates, providing the nutrients that could be absorbed by the plant. In addition, it could be hypothesized that increased soil moisture in the upper layers may maintain nutrients available to plant for longer periods (Prieto *et al.* 2011). Burgess *et al.* (1998) found that water may also move down-wards, or laterally depending on whether dry or moist soil conditions exist in the soil profile. This is the process of hydraulic redistribution

1.4 Measurement of soil water uptake

Measurement and interpretation of water use in agroforestry systems are more complex compared to monoculture studies. Quantifying the partitioning of water use in agroforestry systems is a difficult task due to the rooting environment being highly heterogeneous. Thus a high number of samples are needed to obtain a reliable estimation of water use (Ong *et al.* 1996).

According to Ong *et al.* (1996), three broad approaches can be adopted to determine water use by components of mixed communities:

1. Estimation of transpiration of each component using a transpiration model based on light interception by each component. This approach was used in the Penman-Monteith methodology to calculate transpiration of each component and the radiation interception has to be accurately estimated. The model requires input variables of above-canopy net radiation, temperature and vapour pressure deficit.
2. Total community water use and transpiration by one component may be measured, leaving transpiration by the other component to be estimated by the difference. This approach uses the total water use of the system which is determined by soil water balance. The transpiration of the tree or grass can be obtained using several techniques such as sapflow meters, lysimeters, diffusion porometry, small chamber gas analysis systems or deuterium labelling.
3. Transpiration by each component may be measured separately. In this approach, the water use by each component is calculated separately.

Each approach has advantages and disadvantages such as cost, difficulties in sampling and reliability of the data. According to Ong *et al.* (1996), the most realistic approach is the second option due to the others being technically too demanding, labour-intensive or too expensive to allow a reliable and direct measurement of transpiration for both trees and grass across all seasons.

A clear example of the successful application of water balance for estimation of the water use in agroforestry systems was described by Eastham *et al.* (1988) who researched the effect of tree densities in silvopastoral systems in Queensland, Australia. Using a systematic Nelder fan design (Nelder, 1962), *Eucalyptus grandis* was planted at nine different densities from 42 to 3,580 trees ha⁻¹. Three densities were selected for detailed study: 82 (low), 304 (medium) and 2,150 trees ha⁻¹ (high). The soil water content was periodically measured using neutron probe access tubes to a depth of 5.6 m which were located at different distances from the tree, which allowed determination of the vertical and lateral patterns of water extraction. Total community water use was calculated and the transpiration of the grass component was quantified by lysimeters. Therefore, tree transpiration was estimated by subtraction of the water used by grass from the total community water use. During the 2 year period, water use in both years was highest in summer compared to the winter season. At high tree density, transpiration was close to open pan evaporation during wet periods in 1985, but was considerably below open pan evaporation in 1986 due to lower precipitation.

Another methodology to determine tree transpiration is the sap flow technique which is a reliable and non-destructive measurement of transpiration by trees. It is highly recommended for estimating water use in agroforestry systems. This methodology will be explained in more detail.

Methods to determine soil water content

Soil moisture content is a key component in water balance and is usually measured by the gravimetric method in the surface horizons, where the water content of soil can be determined from the moist and dry weights of samples. The method does not require expensive instrumentation but it is destructive and time consuming (Fernandez *et al.* 2000). For deeper horizons, another method is recommended called 'Neutron Scattering' which requires the use of a neutron probe, an instrument with a source of fast neutrons and a detector of slow neutrons (Gardener and Kirkham, 1952). This method is less time consuming, less destructive and not as laborious as the gravimetric method. The neutron moisture meter (NMM) emits a radiation source of fast neutrons which are reflected back at low speeds to a detector in proportion to the water molecules in the soil. With an appropriate calibration against gravimetric measurements of soil water content, the NMM measurements can be used to provide accurate, non-destructive measurements of water content across the soil profile. Also, water uptake from specific soil profiles can be calculated and by addition, total water of the entire profile can be measured. However, the main disadvantage is the high cost of the neutron probes and the emission of neutron radiation (Fernandez *et al.* 2000).

New methods have been developed in the last decades such as the dielectric methods, where the water content and electrolyte concentration of a soil can be accurately determined from measurement of its dielectric properties. The principle of this method is based on the measurement of the capacitance of the soil, depending on the fact that water has a much higher dielectric constant than either air or the dry constituents of soil. Another methodology is the time domain reflectometry (TDR) which is based on the propagation of high frequency electromagnetic waves through the soil. This method has the same order of accuracy and reproducibility as nuclear methods, and usually does not require site-specific calibration (Wraith and Baker, 1991).

1.5 Sap flow measurement

The most direct method available and widely used for quantification of uptake of water by individual trees is the measurement of sap flow. This method provides direct, continuous and non-destructive measurement of transpiration in the field under real environmental condition.

Several methods are available (Smith and Allen, 1996) which can operate with the principle of *heat balance* (Sakuratani, 1981), *heat pulse* (Green and Clothier, 1988) and *heat probe* (Granier, 1987). The heat ratio method (HRM) theory was detailed by Burgess *et al.* (2001) but in summary this method measured the rate of temperature increase between an upper and lower thermistor after a central needle has released a heat pulse of temperature. Stainless steel needles are inserted in vertical arrangement with in the sap stream, in parallel and equidistant at 5 mm apart. Forster (2012) described the following procedure made by HRM sap flow devices. First an initial temperature measurement is made on the downstream and upstream needles; then a 2.68 seconds 20 J pulse of heat is fired along the central heater needle, there is a 60 seconds wait and then temperature changes are subsequently measured for 40 seconds, finally an average is taken and the change in ratio of downstream versus upstream temperature is recorded. This ratio is proportional to heat velocity (vh , cm hr⁻¹; Marshall 1958) in fresh wood. With collection of additional information such as sapwood depth, bark width, sapwood fresh weight, dry weight, sapwood fresh volume and thermal diffusivity, vh is converted to sap flow rate expressed as Q (m³ hr⁻¹ or d⁻¹). A detailed description of the HRM was reported by numerous authors (Burgess and Dawson, 2004; Dawson *et al.* 2007; Macfarlane *et al.* 2010, Pfautsch *et al.* 2010; Pfautsch *et al.* 2011 and Forster, 2012). Some authors recommended cross-calibration of the heat balance approach against other absolute quantitative techniques.

In addition, sap flow can measure individual roots in order to provide insight into uptake from different zones in the soil. Another technique for assessment of sources of water by plants is the use of variation in the natural abundance of stable isotopes of water (²H and ¹⁸O) in the soil profile, and in rainfall and groundwater, which can provide a means of tracing sources of water used by plants (Smith *et al.* 2004).

1.6 Root measurement

The understanding of root distribution and water uptake in the field provides a basis for the study of root interactions in the soil and between associated plant species (Schroth, 2003). There is no absolute and unique link between particular root properties and functional objectives, thus it is necessary to measure a number of parameters to obtain a full understanding (Atkinson, 2000).

There are several methods available for measurement of roots (Böhm 1979; Schroth 2003; van Noordwijk *et al.* 2004) such as excavation, monolith, auger or coring, profile wall, glass wall, indirect, container, pinboards, or use of isotopes. Most are destructive and require separation of roots from the soil, which are commonly washed (Box 1996). A new generation of methods use NMR (nuclear magnetic resonance) imaging and tomography, rhizotrons, mini-rhizotrons and in-growth bags. This group has the advantage of assessment of change with time.

1.7 Effect of tree densities on above- and below-ground interactions

As mentioned before, an understanding of how tree/grass combinations utilize available resources is fundamental for the successful design of agroforestry systems. Planting arrangement, tree densities, species and management strategies have to be carefully considered. Trees and grasses are in a dynamic above- and below-grass interactions and plant density alters the balance of competition and facilitation between species. Different studies of leucaena plant density and spatial arrangement have been done with the aim to

quantify biomass yield (Cooksley and Goward, 1988). However few studies have focused on how the interaction changes with leucaena density, and these were mainly done in alley cropping systems. Imo and Timmer (2000) evaluated six treatments consisting of three alley spacing widths (2.4 and 8 m) and two within-alley spacing (0.5 and 1 m). Therefore, the following densities were tested: 1,250, 2,500, 5,000 and 10,000 trees ha⁻¹. They found that 4 m alleys improved crop productivity, while competition was higher in the 8 and 2 m alleys. The higher maize crop productivity in the 4 m alleys was associated with increased N uptake, presumably from higher N mineralized from mulch of leucaena. However, close 2 m alleys increased soil fertility but resulted in higher tree competition for moisture and light. Meanwhile, 8 m alley did not have significant effects on soil fertility but increased competition for N.

Detailed information about how tree density of leucaena affects light, water and nutrient competition and facilitation has to be done for a better understanding of the system and to provide practical recommendations to graziers. Different designs have been proposed to quantify the interaction between trees and grasses/crops at a range of tree density.

1.8 Experimental designs to evaluate tree densities in agroforestry systems

Agroforestry systems are more complex than mono-cropping systems in terms of interactions among components and physical characteristics of tree component such as growth (slow or high) or area of influence. Long term effects complicate the design of experiments for these systems (Jaggi *et al.* 2003). The design of tree arrangements and grass/crop components will depend on the interaction to be evaluated. The experimental designs used in agroforestry systems vary from randomized designs (randomized complete design or split plot design) to systematic designs. Huxley (1985) summarized the advantage of use of systematic designs for field experiment compared with conventional designs. He highlighted that systematic designs used smaller areas than randomized block or conventional layouts, used fewer tree plants, and the effective experimental area was greater than conventional configurations. The range of levels of the experiment can be greater than with conventional experiments and can incorporate extreme treatments and finally they can provide observable response treatments that are useful for field demonstrations. However, the drawbacks of these designs are: the systematic design requires a greater level of skill to lay-out in the field, and each plot must be located in an area that is environmentally uniform. These designs need consistent care and attention (e.g. weed control), and although there is adequate replication, the data are basically evaluated by regression analysis. Among systematic designs, the most used to test the effect of tree densities is the Nelder fan design (Nelder, 1962) which allows testing of multiple tree densities in a single plot. Nelder (1962) presented four systematic designs, which differ in shape and potential growing space. However, the most used layout was the circular design which maintains a fixed space between trees that increase with radius length (Parrot and Lhotka, 2012). A successful example of tree/pasture interaction at a range on tree densities using Nelder fan design was done in southeast Queensland, Australia (Eastham and Rose, 1988, Cameron *et al.* 1989; Eastham and Rose, 1990; Eastham *et al.* 1990). Under the STAG project (Soil, Trees and Grass), a Nelder fan experiment was established to investigate above- and below-ground competition between *Eucalyptus grandis* and *Setaria sphaceolata* cv. Nandi at the Samford Pasture Research Station, Brisbane, Australia. The main results from their study were that trees and pasture can be grown together and that thinning regimes would be required to maintain optimum balance between the two components in the agroforestry system. In addition, pasture productivity was greater at intermediate tree density during the first year and it was associated with a higher water use efficiency compared to that found at low and high tree density. Tree root systems were deeper and denser at high densities, and shallower at low tree densities. The experimental design allowed study of the water balance at different tree densities.

1.9 Conclusions

Leucaena-grass pasture systems are profitable and sustainable in northern Australia. The optimal configuration of the leucaena-grass combination is not well understood and a better understanding of above- and below-ground interactions between tree and grass components is needed. These interactions are dynamic, highly variable in space and time. Understanding of above- and below-ground interactions and their impact on light, water and nutrient use efficiency is required. Research is necessary to obtain an holistic view of the leucaena-grass system, to fill these gaps in knowledge and to provide practical recommendations to graziers.

2 Project objectives

The project objectives were:

- 2.1 Measure root architecture and soil water extraction patterns of the leucaena and grass components of pasture, including the impact of within-hedgerow leucaena plant density.
- 2.2 Determine the impact of leucaena hedgerow spacing, and associated competitive interactions, on partitioning of total water use between leucaena and grass.
- 2.3 Determine the ability of leucaena and grass to extract soil water from different depths in the soil profile.
- 2.4 Quantify seasonal changes in leucaena and grass evapotranspiration crop factors.
- 2.5 Evaluate the impact of degree of defoliation of leucaena and inter-row grass on rates of recovery of plant water use and forage production.
- 2.6 Provide management recommendations for hedge-row system design and grazing management that will reduce establishment costs and optimise forage productivity for varying levels of soil water availability in representative agroclimatic environments.

3 Water use, root activity and deep drainage within a leucaena-grass pasture: a case study in southern inland Queensland, Australia

3.1 Introduction

Intensive production systems such as *Leucaena leucocephala* (leucaena)-grass pastures are the key to enhancing profitable cattle production in northern Australia. With an area greater than 200,000 ha in Queensland, leucaena-grass pastures have been shown to be productive, profitable and sustainable (Shelton and Dalzell, 2007). Furthermore, irrigation of leucaena can increase beef production by 3–6 times compared with dryland plantings (Shelton and Dalzell 2007).

Over the past decade, coal seam gas (CSG) exploration in southern Queensland has expanded rapidly, generating a large amount of water as a by-product of the gas extraction process, which must be put to beneficial use. Irrigated systems, capable of using large volumes of water with minimal risk impact on natural aquifers, are needed.

The decision by CSG companies to irrigate leucaena combined with Rhodes grass (*Chloris gayana*) was based on the hypothesis that the roots of trees and grass occupy different soil strata when growing in association (Schroth, 1999) and are capable of maximizing water use in the profile and minimizing deep drainage. In the case of leucaena-grass pasture systems, there is limited information concerning root distribution and water uptake. According to Poole (2003) and Radrizzani (2009), approximately 60% of root biomass of a leucaena-grass pasture was concentrated in the top 0.4 m of the soil profile, with root abundance decreasing rapidly at greater depths, although some roots reached a depth of 6 m under 5–10-year-old leucaena. However, other studies have reported maximum root depth at only 2.8 m in 28-month-old leucaena (Dhyani *et al.* 1990) and at 2.6 m in 38-year-old leucaena in alley cropping with pasture (Radrizzani, 2009) in soils with physical restrictions. Both of these studies reported a restrictive rock layer at these depths, which prevented leucaena from exploring deeper into the regolith.

Technologies for soil water monitoring have advanced over the past decade. EnviroSCAN (Sentek Pty. Ltd., Stepney, South Australia) capacitance systems accurately measure soil water content for irrigation management in Australia and other countries by measuring the electrical constant of the soil (Jabro *et al.* 2005). Precise measurements of soil water are critical for a better understanding of water use by crops and pastures and for irrigation scheduling. For instance, water management can be used to prevent or promote flushing of excess soil salt via drainage below the rooting zone.

Accordingly, as a prelude to a formal program of research, this study was designed to monitor soil water extraction under leucaena-grass pasture using EnviroSCAN to provide background information on: (a) the maximum depth of water extraction (and by inference root activity); (b) the amount and pattern of water extraction of a leucaena-Rhodes grass pasture; and (c) the likelihood of deep drainage below 4 m depth.

3.2 Materials and methods

3.2.1 Site details

Moisture usage was monitored at Santos' Fairview gas field north-east of Injune, Queensland (25°44'40" S, 149°3'19" E), where 234 ha of *Leucaena leucocephala* ssp. *glabrata* and *Chloris gayana* was being irrigated using desalinated CSG water under 4 centre-pivot irrigation systems. The leucaena (cvv. Wondergraze and Tarramba) was sown in November 2009 in twin rows (1 m apart) with 8 m spacing between the centres of the paired hedgerows. Oats, ryegrass and Rhodes grass (cv. Finecut) were sown between the leucaena twin rows in March-April of 2010 but from 2011 onwards, the alley-ways between the leucaena twin rows were dominated by Rhodes grass. The soil types were Black and Red Vertosols (Isbell, 1996), and at all locations the soil profile was >2 m depth to the C horizon and 3–4 m to regolith (substrate).

The subtropical climate has an annual rainfall of 628 mm and average maximum and minimum temperatures of 33.6 and 19.6 °C, respectively, in the hottest month (January) and 20.1 and 3.2 °C in the coolest month (July) (Bureau of Meteorology 2014). An automatic weather station recorded daily rainfall, maximum and

minimum temperatures, wind speed, total radiation and potential evapotranspiration (PET) using the Penman-Monteith equation (Allen *et al.* 1996).

3.2.2 Soil water measurements

Volumetric soil water content was monitored at 4 sites using 8 EnviroSCAN probes connected to dataloggers (RT6 logger, Sentek Pty. Ltd.) with a sampling interval of 15 minutes. Each EnviroSCAN probe had 7 capacitance sensors located at 0.1, 0.3, 0.6, 1.2, 2, 3 and 4 m below-ground level and data were collected over 757 days from August 2011 to August 2013. Four probes were positioned within the leucaena twin rows and 4 probes within the Rhodes grass inter-row sward at 2 sites, 2 and 4 m from the center of the leucaena twin rows. Field capacity point (FC) and wilting point (PWP) were estimated using Irrimax 9.1.1 software tools (Version 9.1.1, Sentek Pty. Ltd.). Total plant-available water (PAW) was calculated from the difference between FC and PWP (Fig. 3.1).

The sensors were installed following the recommendation of Sentek Pty. Ltd., and an *in-situ* calibration equation was developed for Black vertosol ($SF = 0.039 \theta + 0.363$, $R^2 = 0.55$) and Red vertosol ($SF = 0.021 \theta + 0.251$, $R^2 = 0.65$) according to procedures fully explained in the calibration manual (SENTEK, 2001).

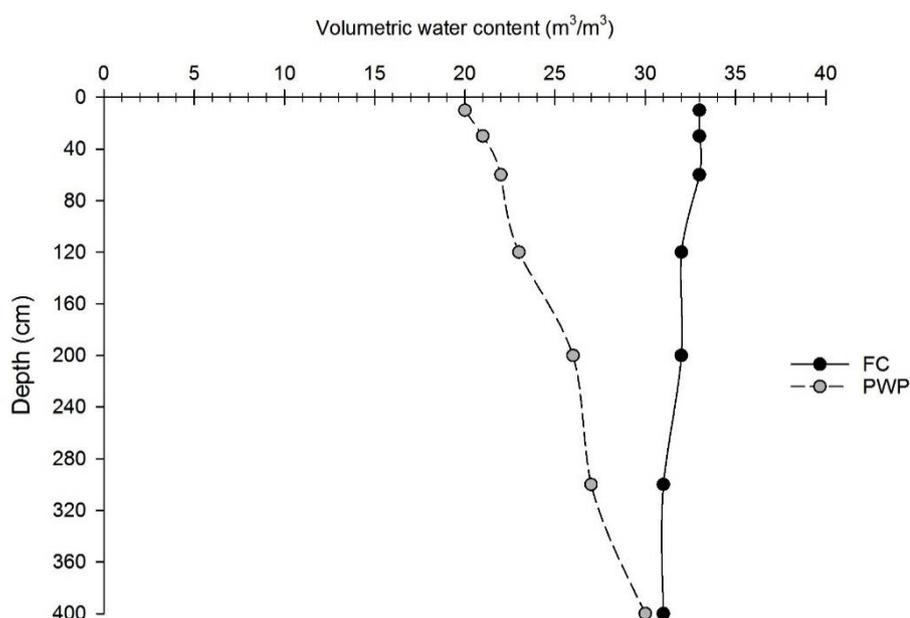


Fig. 3.1: Profile of soil water content used for the study. FC = field capacity; PWP = permanent wilting point.

3.2.3 Depth of water extraction

Depth of water extraction, assumed to be indicative of the maximum depth at which roots were actively taking up water, was estimated using the Irrimax 9.1.1 software tools by measuring the depletion of water in the soil profile during days when no precipitation was recorded. Using the graphing tools of Irrimax 9.1.1, it was possible to observe the activity of roots as defined by daily extraction patterns of >0.1 mm per day. Using this method, it was possible to generate a large database reflecting the extent and depth of water extraction (root activity) per month at each probe.

3.2.4 Water uptake and deep drainage

Decreases in soil water content could be due to evapotranspiration, plant water uptake (WU), runoff (R) or drainage (D). The EnviroSCAN data were used to calculate WU and D for the top 4 m of soil profile from 1 August 2011 to 27 August 2013 at 15-minute intervals. Any change in soil water content between 18:00 and 06:00 h was assumed to be drainage, as evaporation and plant uptake were assumed to be negligible during the night (Ward *et al.* 2014). Runoff was minimized by the high ground cover of the pasture but could not be estimated by the EnviroSCAN probes.

Daily water use (mm d^{-1}) at different depths (0.1, 0.3, 0.6, 1.2, 2, 3 and 4 m) was calculated using IrriMAX 9.1.1 software. Daily WU for the whole profile was obtained by interpolation between sensors. Deep drainage (mm) below 4 m depth was estimated for all probes.

3.2.5 Statistical analyses

A total of 72,635 data points was logged for each probe, totalling 581,080 data points during the 757 days of study. Basic statistics were used to compare depth of water extraction, soil water extraction and deep drainage below 4 m depth data and averages and standard errors were calculated for these parameters plus potential evapotranspiration. Within leucaena twin rows, the averages for probes 1–4 ($n=4$) were used; within the grass inter-row, the data for probes located 2 and 4 m from leucaena twin rows were pooled ($n=4$). Data were pooled for the soil types as there were no differences in water use.

3.3 Results

3.3.1 Site information

A total of 552 mm rain was recorded during the first growing season (October 2011–May 2012), and only 338 mm during the second growing season (October 2012–May 2013) (Fig. 3.2a). Rainfalls during the cool dry seasons (June–September) were 55, 149 and 7 mm for 2011, 2012 and 2013, respectively. (Note: There was an unseasonably high rainfall event of 122 mm during the month of June 2012). The average monthly maximum and minimum temperatures for the growing seasons were 30.1 and 15.6 °C, respectively; values for the cool dry seasons were 21.3 and 5.4 °C. The average values for potential evapotranspiration (PET) were 4.5 and 5.3 mm/d for the first and second growing seasons, respectively. PET for the cool seasons was similar in 2011, 2012 and 2013 with an average of 2.9 mm/d.

Supplementary irrigation was applied from the beginning of the study period but ceased due to lack of available water in April 2012 for probes 1, 3, 4, 5 and 6 and in July 2012 for probes 2, 7 and 8, when 155 mm had been applied (Fig. 3.2a). Grazing commenced in late 2010, 12 months after planting. Initially the pastures were rotationally grazed and cattle were moved to allow at least 50 days recovery. In February 2012, all leucaena was pruned to a height of 0.5 m above the ground to control excessive height and thereafter was continuously grazed.

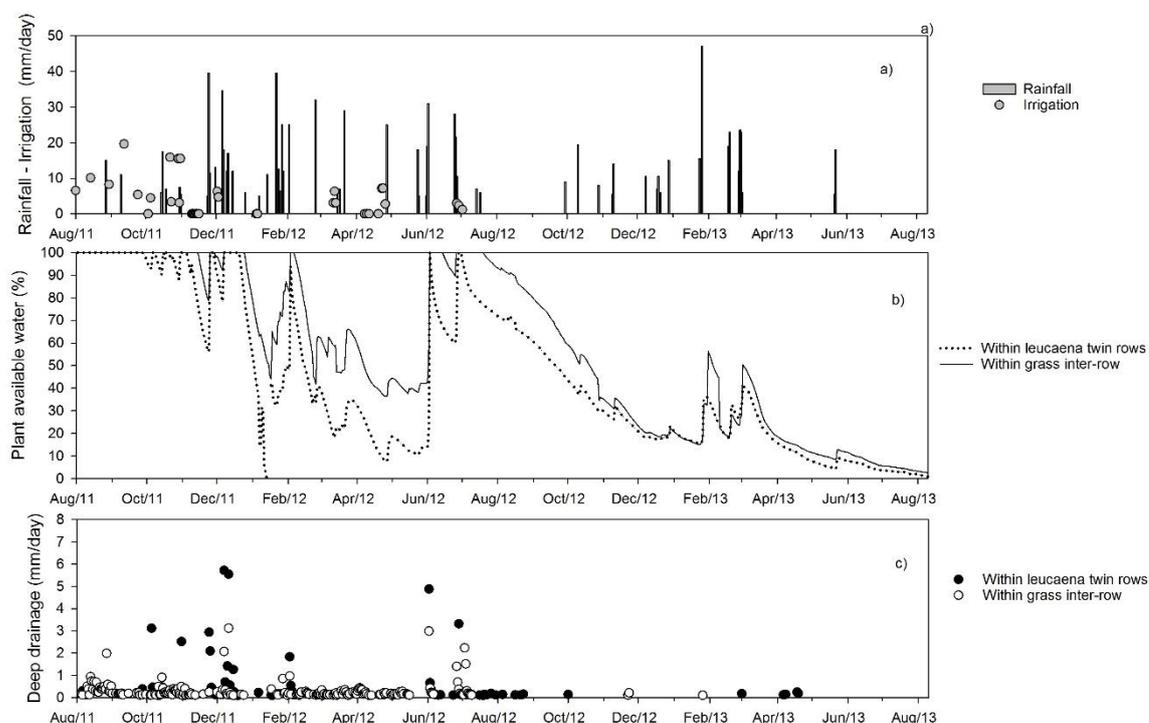


Fig. 3.2: a) Rainfall and irrigation events; b) percentage of plant available water within leucaena twin rows and within the grass inter-row; and c) average daily deep drainage >0.1 mm/d within the leucaena twin rows and within grass inter-row during the period of study.

3.3.2 Soil water content and plant available water

Over the 2 years of the study, the average stored soil water (0–4 m depth) within leucaena twin rows and within grass inter-rows varied from 1,244±7 to 940±41 mm. The average values for field capacity and wilting point were 1,168 and 937 mm, respectively. Thus, regardless of location, relative plant available water (PAW) varied from 100% in August 2011 to 1% in August 2013 (Fig. 3.2b). The unusually high rainfall event in June 2012 refilled the soil profile; however, thereafter PAW decreased due to lack of rainfall and irrigation.

3.3.3 Depth of water extraction

Overall, depth of water extraction was deeper in the growing seasons than in cool dry seasons, regardless of probe locations (Fig. 3.3a and 3.2b). In the first growing season, water extraction within leucaena twin rows (leucaena-dominant) extended to an average depth of 2.2±0.15 m (maximum depth of water extraction was 4 m) (Fig. 3.3a). During the second growing season, depth of water extraction reached 1.9±0.20 m (maximum rooting depth was 4 m). Average depths of water extraction within the grass inter-row (Rhodes grass-dominant) during the first and second growing seasons were 1.8±0.15 and 1.2±0.9 m, respectively, while maximum depth of water extraction within the grass inter-row was 3.5 m (Fig. 3.3b). Depth of water extraction was less than 0.9 m for both pasture types in the cool dry seasons (Fig. 3.3b).

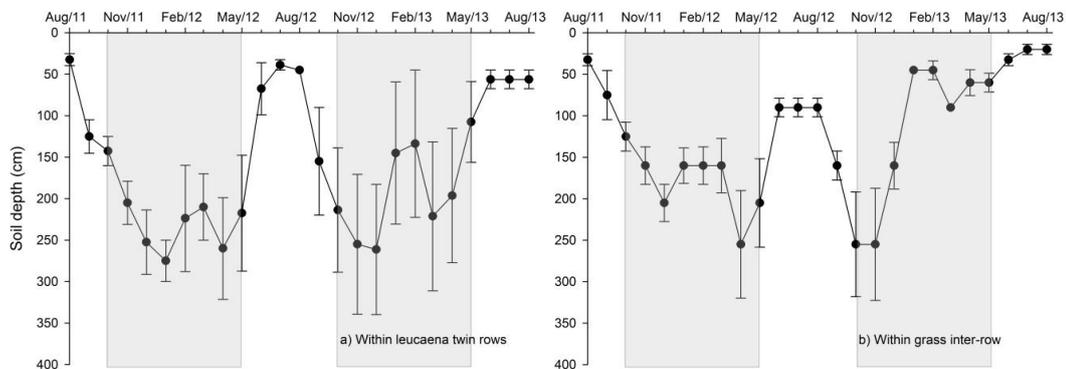


Fig. 3.3: Monthly maximum depth of water extraction detected with IrriMax 9.1.1 software: a) within leucaena twin rows; and b) within the grass inter-row. Growing seasons are shown in light grey and standard error by bars ($n=4$).

3.3.4 Temporal and spatial patterns of water extraction

In general, greatest water extraction occurred in the first wet season. In all seasons, water extraction was highest in surface soil zones, and reduced with depth (Fig. 3.4).

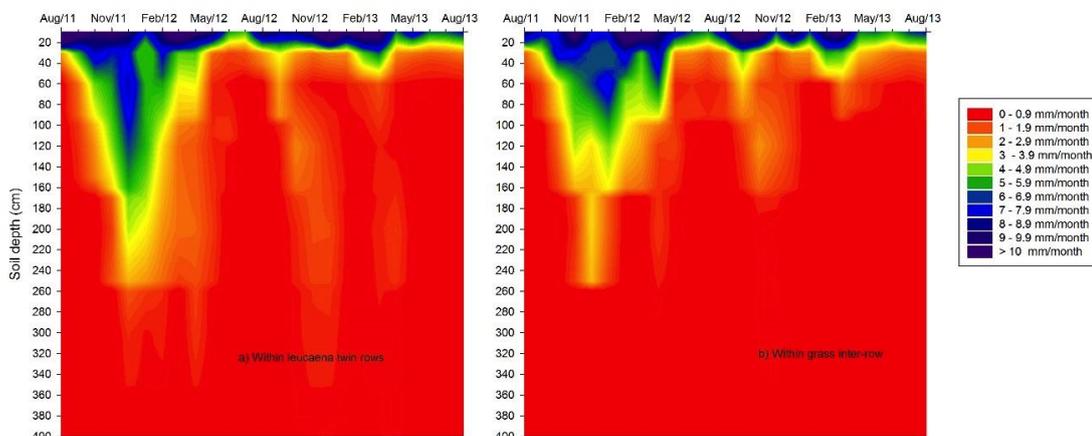


Fig. 3.4: Patterns of average water extraction: a) within leucaena twin rows; and b) within the grass inter-row per 0.1 m soil layer from August 2011 to August 2013. The monthly amount of water extracted per layer is expressed by different colors (mm/month).

During the first growing season, total WU within leucaena twin rows (probes 1–4) was 675 ± 181 mm; however, average WU was higher for probes 1 and 2 at 916 ± 280 mm. An average (probes 1–4) of 77% of water was extracted from surface soil to 1.5 m depth, increasing to 99% for 1.5–3 m depth (Fig. 3.4a; Table 1). During the second growing season, WU was lower at 303 ± 61 mm, of which 75% was extracted from surface to 1.50 m depth, increasing to 94% for 1.5–3 m depth. During the cool dry seasons, the total WU within leucaena twin rows during 2012 was 81 ± 16 mm, reducing to 40 ± 8 mm in 2013, of which 100% was extracted from surface to 1.5 m depth (Table 3.1).

Table 3.1: Accumulated total water extraction per layer and total deep drainage below 4 m depth within leucaena twin rows and within the grass inter-row during the growing and cool dry seasons of 2012 and 2013. Standard errors are presented in italics.

Depth (m)	Average total water extraction per season (mm)											
	Within leucaena twin rows (probes 1–4) (n=4)				Within leucaena twin rows (probes 1–2) (n=2)				Between leucaena twin rows (probes 5–8) (n=4)			
	1 st GS* (304 days)	1 st CDS [†] (122 days)	2 nd GS* (243 days)	2 nd CDS [†] (88 days)	1 st GS* (304 days)	1 st CDS [†] (122 days)	2 nd GS* (243 days)	2 nd CDS [†] (88 days)	1 st GS* (304 days)	1 st CDS [†] (122 days)	2 nd GS* (243 days)	2 nd CDS [†] (88 days)
0–0.5	256 ±58	62 ±16	181 ±25	38 ±8	339 ±80	87 ±19	223 ±14	42 ±18	322 ±66	113 ±18	196 ±8	65 ±19
0.5–1	152 ±34	12 ±3	21 ±7	2 ±2	191 ±62	10 ±6	21 ±2	0	163 ±37	28 ±4	35 ±35	0
1–1.5	111 ±29	4 ±3	25 ±9	0	152 ±25	1 ±1	31 ±6	0	131 ±18	7 ±3	23 ±23	0
1.5–2	75 ±26	1 ±1	23 ±10	0	111 ±37	0	25 ±11	0	40 ±8	1 ±1	5 ±2	0
2–2.5	56 ±22	0	21 ±10	0	83 ±37	0	20 ±11	0	16 ±9	0	1 ±1	0
2.5–3	15 ±12	0	13 ±9	0	25 ±15	0	8 ±4	0	1 ±1	0	1 ±1	0
3–3.5	10 ±7	0	11 ±8	0	15 ±7	0	6 ±3	0	0	0	1 ±1	0
3.5–4	0	0	8 ±7	0	0	0	0	0	0	0	0	0
Total	675 ±181	81 ±16	303 ±61	40 ±8	916 ±280	97 ±30	334 ±99	42 ±14	673 ±107	149 ±21	262 ±23	65 ±19
DD [‡] (mm)	32 ±9.4	11 ±4	5 ±1.4	2 ±1	43 ±7.6	17 ±5.0	8 ±2.3	2 ±0.3	39 ±9.4	16 ±2.5	7 ±1.6	2 ±0.40
R [§] (mm)	552	149	338	7	552	149	338	7	552	149	338	7
IR (mm)	126	26	0	0	103	26	0	0	103	26	0	0
Δ SWC [#]	248	-62	74	15	278	-109	130	22	248	-45	112	25

*GS: growing season; [†]CDS: cool dry season; [‡]DD: depth drainage; [§]R: rainfall; ^{||}IR: irrigation; and [#]Δ SWC: change in soil water content

During the first growing season, WU within the grass inter-row of probes 5–8 averaged 673 ± 107 mm (Fig. 3.4b; Table 3.1). However, the spatial patterns of water uptake were different from those within leucaena rows, with 92% of water extracted from surface to 1.5 m depth. During the second growing season, total water extracted was greatly reduced to 262 ± 23 mm, with $89 \pm 5\%$ extracted to 1 m depth. During the first cool dry season, average total water uptake was 149 ± 21 mm (Table 3.1), with 97% extracted from surface to 1.5 m depth. During the second cool dry season, total water uptake was lower at 65 ± 19 mm, with 100% of water being extracted from surface to 0.5 m depth (Table 3.1).

3.3.5 Deep drainage below 4 m depth

Deep drainage below 4 m for the study period was 50 ± 12.5 and 64 ± 15.4 mm for the leucaena and grass inter-row, respectively. This is 4.1 and 5.4% of total rainfall plus irrigation.

It was greatest when significant rainfall events occurred when moisture content of soil profile was near FC (Fig. 3.1a and 3.1b; Table 3.1). Thus highest deep drainage occurred when rainfall events refilled the soil profile to more than 1,200 mm, i.e. $\geq 100\%$ PAW (Fig. 3.1a and 3.1c). Deep drainage within leucaena twin rows was 31.5 ± 9.4 mm during the first growing season, but lower at 4.5 ± 1.4 mm during the second growing season. In the first cool dry season of 2012, deep drainage was 11.1 ± 4 and 1.8 ± 1 mm during the cool dry season of 2013.

Within the grass inter-row during the first and second growing seasons, deep drainage volumes were 38.7 ± 9.4 and 6.6 ± 1.6 mm, respectively. These volumes were similar to the 43 ± 7.6 and 8.3 ± 2.3 mm of deep drainage registered for probes 1 and 2 located within leucaena twin rows. By comparison deep drainage volumes within the grass inter-rows during the cool dry seasons were 16 ± 2.5 and 2.4 ± 0.4 mm for 2012 and 2013, respectively.

3.4 Discussion

The motivation for this study was based on the requirement that ground water extractions, as part of the CSG process must be used for beneficial purposes e.g irrigation of agricultural crops and pastures. As CSG water varies in availability from limited to excess volumes, the potential outcomes of such variable irrigation scheduling need to be better understood.

The objective of this study was to monitor and describe the water extraction (and by inference apparent root activity) and deep drainage of an irrigated leucaena-grass pasture grown on Vertosols. The methodology comprised 2 years of detailed monitoring of spatial and temporal patterns of water extraction, and hence root activity, and deep drainage below 4 m depth. Data showed that all parameters varied depending on rainfall events, season and management of the leucaena-grass pastures.

3.4.1 Root activity and water extraction

Depths of water extraction and water uptake patterns, shown so dramatically in Fig. 4, are of particular interest in agroforestry systems as trees and grasses are considered to occupy different soil strata when grown in association (Schroth 1999). In this survey, water extraction was used as a proxy for depth of rooting activity. Maximum depth of water extraction and water use (WU) were modestly greater within leucaena twin rows (leucaena-dominant) than within the grass inter-row (Rhodes grass-dominant). When growing at maximum capacity in the first growing season, water extraction within leucaena twin rows extended to an average depth of 2.2 ± 0.15 m with a maximum depth of 4 m. By contrast, mean depth of water extraction within the grass inter-row was 1.8 ± 0.15 m with a maximum depth detected of 3.5 m. It is unlikely that roots of grass reached 3.5 m depth, and it is possible that lateral roots of leucaena were exploiting soil moisture under the grass inter-row. Further studies are needed, including physical sampling of plant roots, to determine the origin of active roots.

The percentage of total WU within leucaena twin rows below 1.5 m depth was 25% (leucaena-dominant) compared with just 10% between rows (Rhodes grass-dominant). This suggested that there was only a small degree of complementarity in water use between the trees and grass, with leucaena accessing water deeper in the soil profile. Various authors mention that, in successful agroforestry systems, trees can access water

resources that the crop or grass would not otherwise access (Cannell et al.1996; Schroth 1999; Fernandez et al. 2008). This assertion was not strongly supported in this study.

These results confirm those reported by Poole (2003), who found that maximum rooting depth for another tropical grass (buffel grass, *Cenchrus ciliaris*) was 1.7 m in Grey Vertosols in central Queensland, Australia. However, the depth of water extraction and by inference active rooting depth of leucaena observed in this study was much shallower than that reported by Poole (2003), who found physical evidence of roots of 5–10-year-old *L. leucocephala* to 5.9 m depth. Rooting depths similar to ours have been reported at 2.8 m in 28-month-old leucaena (Dhyani et al. 1990), at 2.6 m in 38-year-old leucaena in alley cropping with pasture (Radrizzani, 2009) and at 2 m in an alley cropping system with maize (Rao et al. 1993).

Active water extraction by leucaena was shallower during the second growing season due to the combined effects of lower rainfall, absence of irrigation and severe defoliation by pruning and grazing. This was unexpected as leucaena has a reputation for continuing to grow during prolonged dry periods, when upper layers of the soil profile are dry (i.e. soil water content <PWP); this attribute is often cited as one of its major production advantages (Shelton and Dalzell, 2007). We postulate that the more severe defoliation experienced in the second growing season may have contributed to the lower WU of leucaena during this time. The effects of continuous heavy grazing were also severe on Rhodes grass, as depth of water extraction reduced from 1.5 m to 0.5 m. During the cool dry seasons, the shallow depths of water extraction by both species (0.66 ± 0.18 m) could be attributed to lower temperatures, which would have limited plant growth (Cooksley et al. 1988; Moore et al. 2006).

3.4.2 Water uptake patterns

Water uptake was greatest in the upper soil profile and decreased with depth. This pattern reinforces the findings of Callow (2011), who reported that the capacity of warm season forages to extract soil water generally decreased with depth.

Season had a strong influence on total water extraction, which was highest in the first growing season due to high evapotranspiration demands associated with rapid growth of the pasture and adequate soil water content leading to deeper root exploration by both leucaena and Rhodes grass.

The amount of water extracted during the cool dry seasons was much lower than during the growing seasons as low soil water levels coupled with lower temperatures, as well as defoliation, would have limited plant growth. The influence of defoliation on WU requires further study. Overall, the amounts of water extracted were lower than those reported by Narain et al. (1998) at a location receiving an average of 1,523 mm of rainfall. In a 4-year study of water use under different land uses, which included a leucaena monoculture and a leucaena-grass system, they reported average WUs of 1,528 and 1,397 mm/yr, respectively. They found similar seasonal differences in water extraction between growing and cool dry seasons, with water extraction limited by low available soil moisture and reduced plant growth during winter.

3.4.3 Water use of leucaena versus grass

There was some evidence that leucaena extracted more water than grass alone as its greater depth of rooting made a modest difference in water uptake. Water extracted within the grass inter-row (Rhodes grass-dominant) was 25% lower than that extracted within leucaena twin rows. According to Schroth (1999), while depth of root exploration is important, it is necessary also to consider root distribution and root activity within the soil profile.

3.4.4 Deep drainage below 4 m depth

Although the potential advantages of leucaena-grass systems in controlling deep drainage is hypothesized (Shelton and Dalzell 2007), there are few data on the amount of deep drainage that occurs in leucaena-grass pastures. However, there are considerable data on deep drainage in pasture and native vegetation (Ownes et al. 2004; Silburn et al. 2009; Tolmie et al. 2011). In this study, daily deep drainage below 4 m depth differed between growing seasons and cool dry seasons. Deep drainage was greatest when significant rainfall events or frequent irrigation occurred at times when the soil moisture profile was near field capacity. Thus higher daily deep drainage occurred during the first growing season and the cool dry season of 2012 following an

unseasonal rainfall event. During the late phase of the study, when rainfall and corresponding soil moisture values were much lower, average drainage was low. There was no major difference between deep drainage within leucaena twin rows and within the grass inter-row.

Poole (2003) modelled the probability of deep drainage under leucaena-buffel grass pastures, buffel grass only and annual summer grain (sorghum) cropping over a 100-year period and also found that higher rates of deep drainage were related to higher rainfall events. The model predicted that there would be less deep drainage under leucaena-grass pastures than under buffel grass pastures and grain sorghum annual cropping. In soils without limitation, the probability of annual deep drainage of 50 mm (over a 100-year period) was 85% for annual sorghum cropping, 60% for buffel grass pastures and 20% for leucaena-grass pastures. Robinson *et al.* (2010), using simulation modelling for Goondoola Basin in a semi-arid region of Queensland, found that deep drainage was strongly related to soil type and vegetation; clearing native vegetation and introducing crops and pastures increased deep drainage. Pastures with deeper roots (2.4 m depth), such as leucaena-grass pasture, growing on 6 different soil types had 25 mm less of deep drainage than wheat cropping.

The study period had below average to average rainfall and greater deep drainage would be expected in wetter years and with greater irrigation, although plant growth and water use may also be greater. Modelling of soil water and salt balances of leucaena and grass inter-row systems using data from this study, with various levels of irrigation, is recommended to investigate the risks of deep drainage over an extended climate sequence.

3.5 Conclusions

EnviroScan sensors were a useful tool for characterizing spatial and temporal patterns of water extraction, and by inference root activity of leucaena-Rhodes grass pasture. A marked seasonal pattern of soil water extraction was observed which was greater during growing seasons and lower in cool dry seasons. Both, leucaena and Rhodes grass had greater soil water extraction from the upper soil layers (<1.5m depth) suggesting high levels of competition for water resources between plant species. Low rainfall, defoliation and low temperatures negatively affected depth of soil water extraction and therefore reduced total soil water extraction. There was some evidence that leucaena roots were active slightly deeper in the soil profile than roots of Rhodes grass.

The highest values of deep drainage below 4 m occurred when rainfall events coincided with soil moisture near to 100% PAWC. Therefore, irrigation should be avoided at this time. Deep drainage below 4 m soil depth within leucaena twin rows differed little from that within the grass inter-rows.

Given that soil water usage by both leucaena and Rhodes grass was greatest in the upper layers of soil (<1.5 m), future research should focus on how the level of competitive interaction might be managed by choice of row spacing and frequency of irrigation. Also, additional studies are needed, including: (a) physical soil sampling to determine the depth and distribution of active roots; and (b) how defoliation affects rooting behaviour and water use of leucaena. Modelling of the soil water and salt balances of leucaena and grass inter-row systems using data from this study, with various levels of irrigation, is recommended to investigate the risks and advantages of deep drainage to manage soil salt profiles.

4 Measuring the effect of defoliation on water use of *Leucaena leucocephala*

4.1 Introduction

Leucaena leucocephala [(leucaena) Lam. de Wit ssp. *glabrata* (Rose) Zarate] mixed with grass pasture is widely used in sub-tropical and tropical Queensland Australia due to its highly productive and sustainable characteristics (Shelton and Dalzell, 2007). The multiple benefits of leucaena-grass pastures have been well documented (Dalzell *et al.* 2006; Shelton and Dalzell, 2007; Radrizzani *et al.* 2010a; Harrison *et al.*, 2015; Taylor *et al.* 2016).

The persistence of leucaena under grazing has been reported by many studies of the effect of grazing and frequency of defoliation on regrowth and biomass production (Horne *et al.*, 1985; Duguma *et al.* 1988; Stür *et al.* 1994; Cobbina, 1998 Tudsri *et al.* 2002;). Regrowth of leucaena plants depends on the activity of meristematic tissues, the amount and photosynthetic capacity of residual leaves and the carbohydrate reserves available in the plant after defoliation (Stür *et al.* 1994). Following grazing or cutting, there are three phases which are defined by a sigmoidal curve; the first is a lag phase when regrowth is slow, the second is when leaf production increases markedly and the last phase occurs when older leaves start to senesce (Stür *et al.* 1994).

The common defoliation practice when leucaena is used as fodder in Australia is direct grazing by ruminants (Shelton and Dalzell, 2007). This system is also employed in Colombia and Mexico (Murgueitio *et al.* 2011). Leucaena can also be manually harvested and transported to another location for feeding animals (cut and carry systems). Such systems are widely used in Indonesia and Thailand. Regardless of feeding system employed, leucaena plants are exposed to frequent defoliation. When directly grazed, animals remove mainly leaf and green stems up to ~5 mm diameter, although the proportion of leaf removed and the thickness of stems grazed depends on the stocking rate used and the duration of grazing. In cut and carry systems, larger branches of leucaena are severed and the entire branch is removed. Severe mechanical pruning is also common practice in Queensland where height is controlled every 5-10 years using slashers or purpose built cutters to reduce the height of shrubs to 0.5 m (Dalzell *et al.* 2006).

Although many researchers have studied the effect of defoliation on biomass production, the process of water uptake by leucaena plants is not well understood. Such information is vital for best management of irrigation and limited seasonal water supply. Jackson *et al.* (2000), working with an agroforestry system with *Grevillea robusta* combined with maize, determined that pruning can be a powerful method of controlling water balance in the system. They found that moderate pruning of the tree canopy did not limit water demand; however, when the canopy was heavily pruned, water uptake was significantly reduced and the soil profile was able to recharge following precipitation. Also, a two-year monitoring study of water use of leucaena-grass pasture in southern inland Queensland, Australia (Pachas *et al.* 2016) observed a lower water extraction during the second year which they attributed to lower plant available water and the effect of severe defoliation by pruning and grazing. They recommended further studies of the effect of intensity of defoliation on the water uptake of leucaena plants.

Accordingly, the objectives of this study were (1) to quantify the effect of intensity of defoliation on water use of leucaena under controlled conditions, (2) to compare different approaches to measuring water uptake of leucaena and, (3) to measure leucaena transpiration under field conditions.

4.2 Materials and methods

4.2.1 Experiment 1

A study was carried out in a controlled glasshouse facility at the University of Queensland, St. Lucia, Australia. The objective of the experiment was to measure effect of different intensities of defoliation on water use of leucaena. Twenty-four plants of *Leucaena leucocephala* (Leucaena) cv. Tarramba growing in 330 mm (18 L) ANOVApot® (ANOVApot Pty. Ltd. Brisbane, QLD, Australia) were used. Leucaena seedlings were transplanted

into the ANOVApots on 7 February 2014 filled with soil comprising 68% potting mix, 30% coir, 0.6% gypsum, 0.6% dolomite and 0.8% osmocote.

In this system, leucaena plants were grown without water restriction using a “twinpot” system (Hunter *et al.* 2012; Hunter and Scattini, 2014). This method was used to directly measure leucaena water uptake. A schematic representation of the twinpot system adapted from Hunter *et al.* 2012 summarizes the function of this novel system (Fig. 4.1). Briefly, the twinpot system consisted of two pots; the upper pot supported the leucaena plants growing in potting mix soil, whereas the lower pot contained water. Water moved from the lower to the upper pot by using capillarity tapes installed in both pots (Hunter and Scattini, 2014). The lower pot was connected to an external float valve and this valve was connected through a medical infusion set to a reservoir of 15 litres. Water from the reservoir moved through the polyethylene tube to the lower pot by negative tension maintaining a constant water table in this pot (a layer of 10 mm).

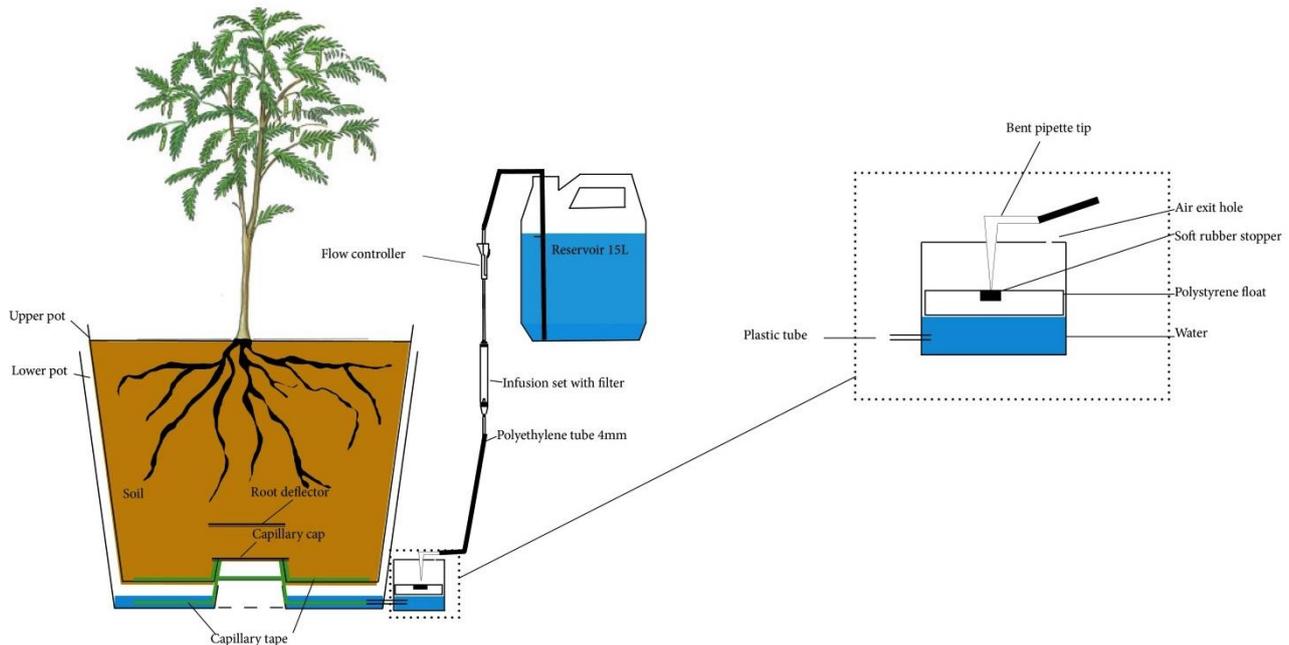


Fig. 4.1: Schematic representation of the twinpot system adapted from Hunter *et al.* (2012) which was used to measure water uptake of *Leucaena leucocephala* at the University of Queensland, Australia.

The experiment commenced in October 2014 when leucaena trees reached an average of 2.5 m height and 0.05 m basal diameter. Pots were arranged in a randomized complete block design with six treatments and four replications. Six intensities of defoliation were applied to the leucaena trees: 1) 0% defoliation, 2) 25% defoliation, 3) 50% defoliation, 4) 75% defoliation, 5) 100% defoliation and, 6) 100% defoliation plus stem cut to 0.75 m height. The defoliation was performed cutting the bi-pinnate leaf of leucaena from the base of petiole with a pruning scissor. The defoliation started from the bottom to the top of the tree, removing one of four leaves (25%), removing alternative leaves (50%), removing three of four leaves (75%) and removing all the leaves from the tree (100%).

Trees were defoliated on 20 October 2014. The experiment was conducted for 32 days from 20 October to 21 November 2014. Measurement of water uptake was done by weighing the reservoir daily. Reservoirs were refilled every 2-3 days according to environmental demand. Leaf area was measured using a portable leaf area meter (Li-3000C, Licor) from the beginning of the experiment. Two calibration equations were fitted between leaf area per compound leaf and length of its rachis to estimate total leaf area per tree after 15 days ($R^2=0.87$, $n=40$) and at completion of the experiment ($R^2=0.90$, $n=40$). Solar radiation ($W m^{-2}$), temperature ($^{\circ}C$), relative humidity (%) were measured and vapour deficit pressure (VPD, kPa) was calculated (Murray 1967).

4.2.2 Experiment 2

In a second experiment in the controlled glasshouse facility, methods of measurement of water uptake were compared, namely: the twinpot system and sap flow meters. Twenty leucaena seedlings were transplanted in October 2014 into a twinpot system following a similar methodology described for experiment 1.

The experiment commenced in February 2015 when trees reached an average basal diameter of 0.05 m and height of ~2.5 m. Ten similar trees were selected and randomly placed in the glasshouse. The physical properties of the trees are shown in Table 4.1. Sap flow meters (SFM, ICT International, Armidale, NSW, Australia) were installed in the main stem at ~0.1 m above pot soil level on each tree. Thus, 10 sap flow devices were used to measure leucaena transpiration ($\text{cm}^3 \text{ tree}^{-1} \text{ day}^{-1}$). Briefly, sap flow meters measure the xylem sap flow using the Heat Ratio Method (HRM) and it is described by Burgess *et al.* (2001) and Forster (2012). Sap flow meters measure heat pulse velocity by obtaining the ratio of downstream sapwood temperature to upstream sapwood temperature following the release of a heat pulse, using three stainless steel needles inserted in vertical alignment within the sap stream. As the ratio is proportional to heat velocity (v_h , cm hr^{-1}) (Marshall 1958) in fresh wood, collection of additional leucaena wood properties such as sapwood depth, bark depth, sapwood fresh weight, dry weight, sapwood fresh volume, and thermal diffusivity is necessary to convert v_h to sap flux density (J_s , $\text{ml mm}^{-2} \text{ sapwood h}^{-1}$) and to volumetric sap flow rate (Q , $\text{m}^3 \text{ h}^{-1}$ or d^{-1}) (Burgess *et al.* 2000; Forster 2012). SFMs were installed in the primary stem of leucaena and were connected to a solar panel which logged every 30 minutes using a heat pulse of 20 joules. During a period of 12 days, from 25 February to 9 March 2015, water uptake of leucaena was measured using the twinpot system and the sap flow meter technique. Similar environmental parameters as for experiment 1 were measured.

Table 4.1: Physical properties of leucaena trees selected for experiment 2.

Number of trees	Diameter (cm)	Total height (m)	Bark width (mm)	Sapwood depth (mm)	Sapwood area (cm^2)
10	3.49 \pm 0.10	2.60 \pm 0.152	0.6 \pm 0.02	10.08 \pm 0.36	7.78 \pm 0.4

4.2.3 Experiment 3

The third experiment was carried out at the University of Queensland, Gatton research farm (27.54°S, 152.34°E). The objective of the experiment was to measure water uptake of leucaena under field conditions. Sap flow of leucaena trees was measured in a Nelder fan experiment (Nelder, 1962). The design of the experiment was described by Pachas *et al.* (2015). Briefly, leucaena cv. Tarramba was planted on 27 November 2013 in twelve concentric rings of trees with radii of 0.6, 0.9, 1.3, 1.9, 2.8, 4.0, 5.9, 8.5, 12.3, 17.9, 25.9 and 37.6 m. The outer and the innermost rings as well as spokes 1, 5, 9 and 13 were used as guards. Each ring contained 16 trees planted equidistant around the circumference, giving a range of tree densities of 100, 210, 442, 928, 1,951, 4,100, 8,618, 18,112, 38,065 and 80,000 trees ha^{-1} . The rate of change along planting spokes (α) used was 1.45 with an angle between spokes (Θ) of 22.47° (0.3992 radians). The total area occupied for the experiment was 0.47 ha with 192 leucaena trees. Rhodes grass cv. Finecut was sown on 11 March 2014 in two quarters of the Nelder fan (Fig. 4.2).

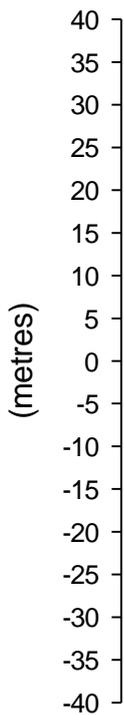


Fig. 4.2: Nelder fan design with 12 radii and 16 spokes. Each point represents a leucaena tree. Inner and outermost rings were guards.

For the experiment, three densities of leucaena (100, 928 and 4,100 trees ha⁻¹) were selected to measure transpiration using the sap flow technique previously described for experiment 2. For each density, 12 trees were selected, six growing without grass competition and six with grass competition (Table 5.2). SFMs were installed in the primary stems of leucaena; each SFM was connected to a solar panel and was logged every 30 minutes using a heat pulse of 20 joules. For each density, daily sap flow rate (cm³ day⁻¹) per stem was calculated using the Sap Flow Tool (ICT International, Armidale, NSW, Australia /Phyto-IT BVBA, Mariakerke, Belgium). To estimate total tree transpiration on multi-stem trees, all primary stem diameters were measured approximately 0.2 m above-ground using a digital calliper. Sapwood area of non-measured trees was estimated and sap flow was calculated based on the linear regression between the sapwood area and sap flow per stem obtained at the same tree density (Doronila and Forster, 2015). To compare sap flow values among different periods, transpiration was corrected by VPD.

Table 4.2: Specification of *Leucaena leucocephala* trees and branch sizes used for sap flow measurements in the Nelder fan experiment. Date refers to time-period when trees in that specific density were measured.

Density (trees ha ⁻¹)	Date	Number of Stems	Basal diameter range (cm)	Bark width (mm)	Sapwood depth (mm)	Sapwood area (cm ²)
100	From 12/03 to 15/03/15	12	1.23 - 7.12	0.65±0.04	10.15±2.4	7.65±2.3
928	From 16/03 to 19/03/15	12	1.33 - 5.43	0.58±0.03	7.4±0.6	3.43±0.74
4,100	From 21/03 to 26/03/15	12	1.06 - 4.35	0.56±0.03	6.79±0.2	2.71±0.36

4.2.4 Statistical analysis

In experiment 1, repeated measurement analysis using the ANOVA general linear model was used to determine the effect of defoliation on daily water use (DWU) and cumulative water uptake of leucaena. The model included defoliation and time and their interactions as fixed factors. The model also included trees as a random effect. Linear regression analysis was used to test the relation between leaf area and water use per tree. In experiment 2, orthogonal regression with error variance ratio of 0.9 was used to determine differences between the twinpot system and sap flow meters for measurement of water uptake of leucaena plants. In experiment 3, linear regression analysis was used to determine the relationship between sapwood and sap flows per stem (cm³ stem⁻¹ day⁻¹). To test whether the sap flow rate per stem differed according to leucaena density and grass competition, sap flow data were corrected for environmental demand (divided by average vapour deficit pressure for period of sampling). Analysis of covariance was carried out using the General Linear Model, and water extraction rate as the variable response. The model included density, grass competition and their interactions, while sapwood area was used as covariant. For all experiments, statistical analyses were carried out using Mintab 16 (Minitab Inc., State College, PA).

4.3 Results

4.3.1 Experiment 1

During the study period, the average daytime solar radiation was 449.5 W m⁻² (Fig. 4.3a), mean daytime temperature was 24.7°C, mean daytime RH was 66.1% (Fig. 4.3b) and average daytime vapour pressure deficit (VPD) was 1.1 kPa (Fig. 4.3c). Maximum temperature reached was 38.1°C on 16 November and short periods of very high VPD occurred on 28 October and 16 November (Fig. 4.3c). Average rate of water extraction is shown in Fig. 4.3d. Plants with high levels of defoliation (100% and 100% + cut) had reduced demand during the first 10 days following pruning; thereafter water uptake rapidly increased and uncut plants reached similar values of water extraction as for other treatments after approximately 20 days. However, leucaena plants that were cut (treatment 6) had the lowest rate of water use for the entire period under study. Plants with 0% and 25 % defoliation had highest water use while plants with 25% defoliation had the highest water uptake from 28 October to 7 November and highest overall water use (Fig. 4.3d and 4.4).

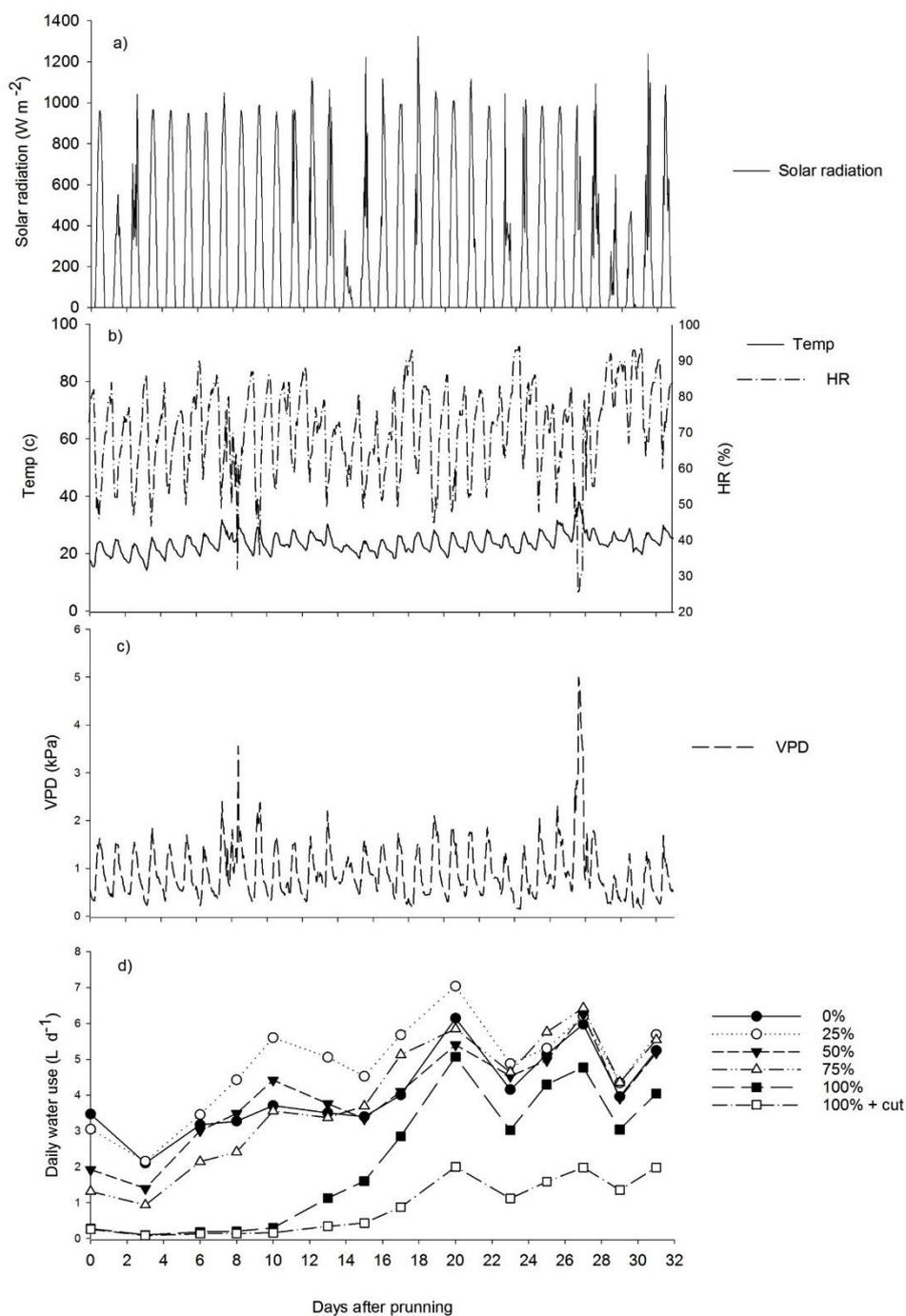


Fig. 4.3: Average daytime environmental conditions measured at the University of Queensland, glasshouse facilities, St. Lucia for experiment 1 from 20 October to 21 November 2014: a) solar radiation (W m^{-2}), b) air temperature ($^{\circ}\text{C}$) and relative humidity (%), c) vapour pressure deficit (kPa), and d) daily water use per tree (L day^{-1}) at six defoliation intensities.

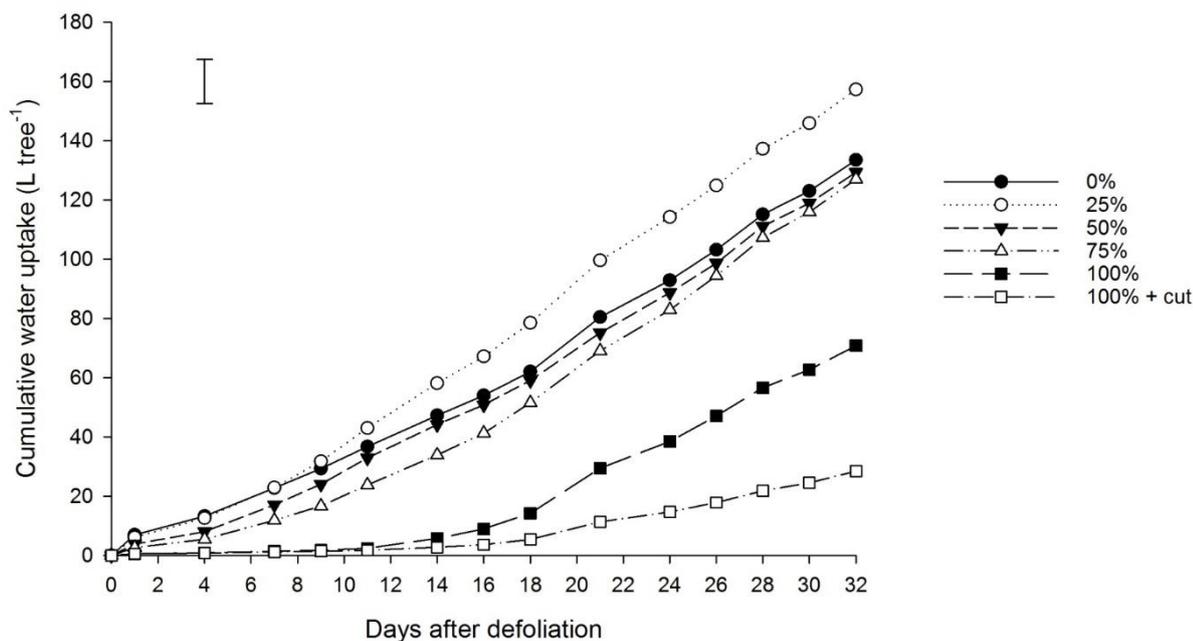


Fig. 4.4: Average cumulative water uptake measured at 6 defoliation intensities using twinpot system. The error bar indicates LSD ($P=0.05$) based on the analysis of all 6 treatments.

There was an interaction between defoliation and time ($P<0.013$). Although, leucaena plants with 25% defoliation had slightly greater cumulative water extraction, there was no significant difference between plants which experienced defoliation intensities from 0% to 75% after 32 days. However, plants with 100% and 100% + cut had significantly reduced water use at 48% and 79% of the control treatment respectively (Fig. 4.4).

Trees with 25% of defoliation had greater average DWU of 4.8 ± 0.3 L tree⁻¹ compared to the other defoliation treatments (Fig. 4.3d and 4.4). Daily water use of leucaena plants without defoliation was 4.1 ± 0.3 L tree⁻¹ day⁻¹. Similar values were observed with 50% and 75% defoliation (4 ± 0.4 and 3.9 ± 0.5 L tree⁻¹ day⁻¹ respectively).

The relationship between daily water use per tree and leaf area was positive ($R^2= 0.82$) (Fig. 4.5).

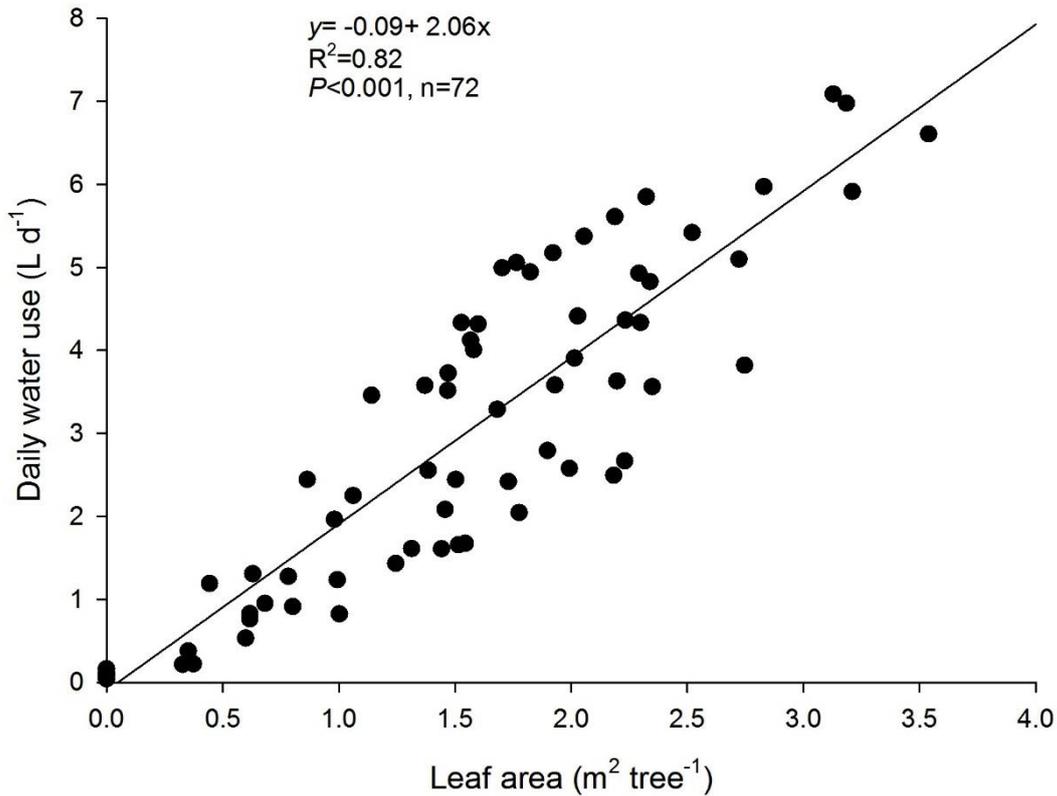


Fig. 4.5: Linear regression between leaf area per tree (m² tree⁻¹) and average daily water use per tree (L day⁻¹) for all the trees evaluated during the period of study.

4.3.2 Experiment 2

For the period of study, the average temperature was 26.4°C, maximum and minimum temperatures were 36 and 22.3°C respectively, average RH was 74.3% and average VPD was 1.23 kPa.

The orthogonal analysis suggested there was no difference in total water used by leucaena whether measured by the twinpot system or sap flow meter techniques (Fig. 4.6a). However, the average water use per tree measured by twinpot system was slightly greater (9.7%) than measured by SFM technique (Fig. 4.6b).

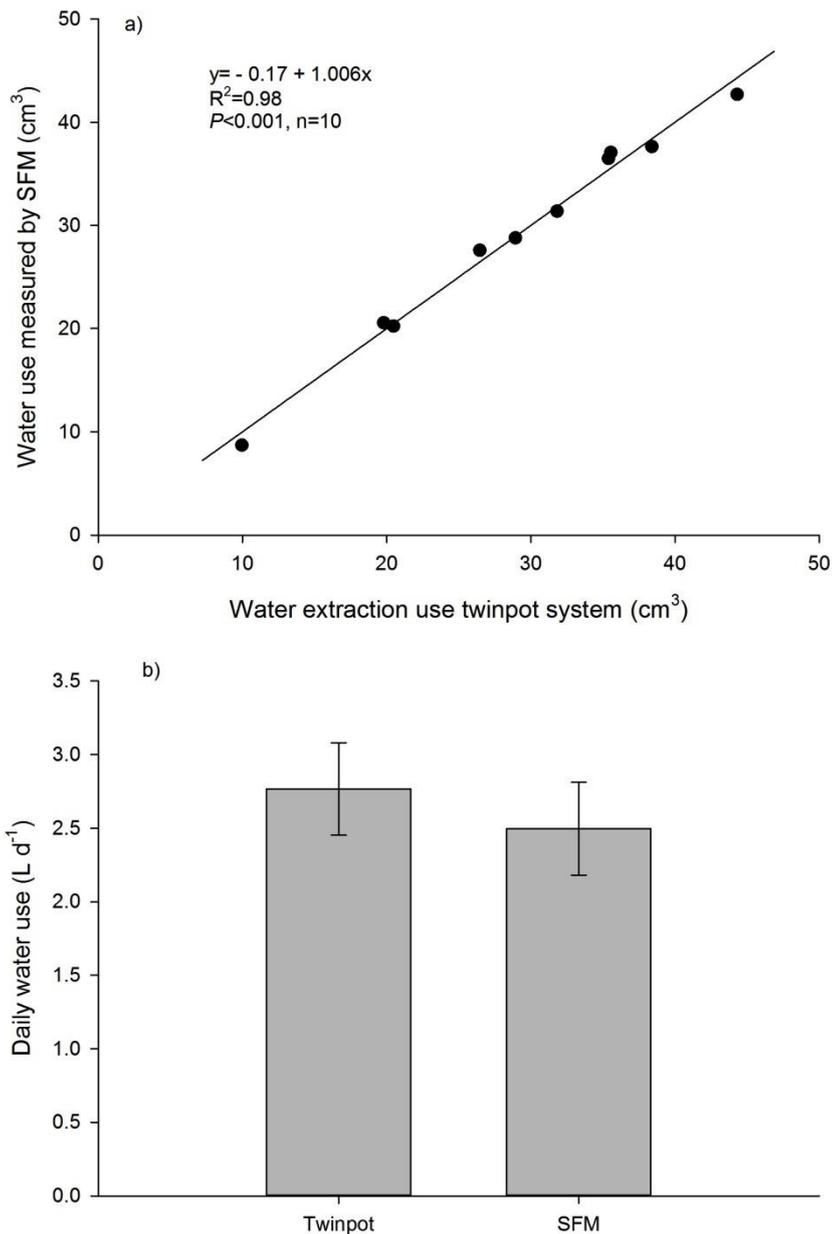


Fig. 4.6: Comparison of water used by leucaena plants using twinpot system and sap flow meters: a) Orthogonal linear regression between water extraction methods and, b) average daily water uptake. Bars indicated standard errors.

4.3.3 Experiment 3

Environmental conditions during the study period were: average temperature 24.9°C ; maximum and minimum temperatures 31.4 and 21.9°C respectively; average RH was 68.4% ; and average VPD was 1.39 kPa (Fig. 4.7). There was no precipitation.

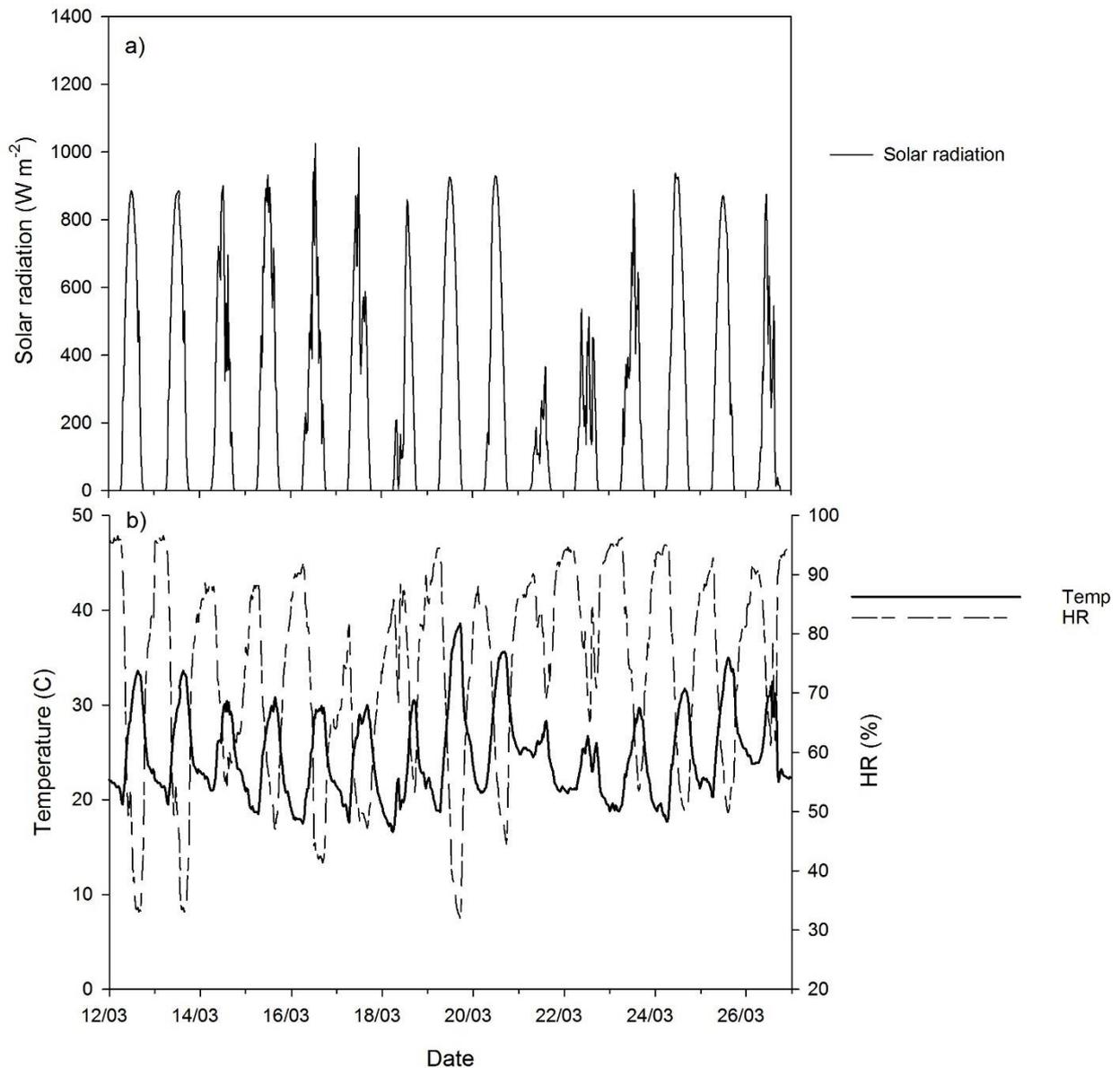


Fig. 4.7: Average daytime environmental conditions measured at Gatton research station for experiment 1 from 13 to 27 March 2015: a) solar radiation (W m^{-2}) and b) air temperature ($^{\circ}\text{C}$) and relative humidity (%).

Analysis of covariance showed that no evidence that density ($P=0.831$) and grass competition ($P=0.684$) influenced sap flow rate ($\text{cm}^3 \text{ day}^{-1} \text{ stem}^{-1}$) which was strongly related to sapwood area per stem ($P<0.001$) (Fig. 4.8). Therefore, sap flow data from different densities and grass competition were pooled and a significant linear regression was obtained ($R^2= 96$, $P<0.001$) between daily water extraction per stem and sapwood area per stem (Fig. 4.8).

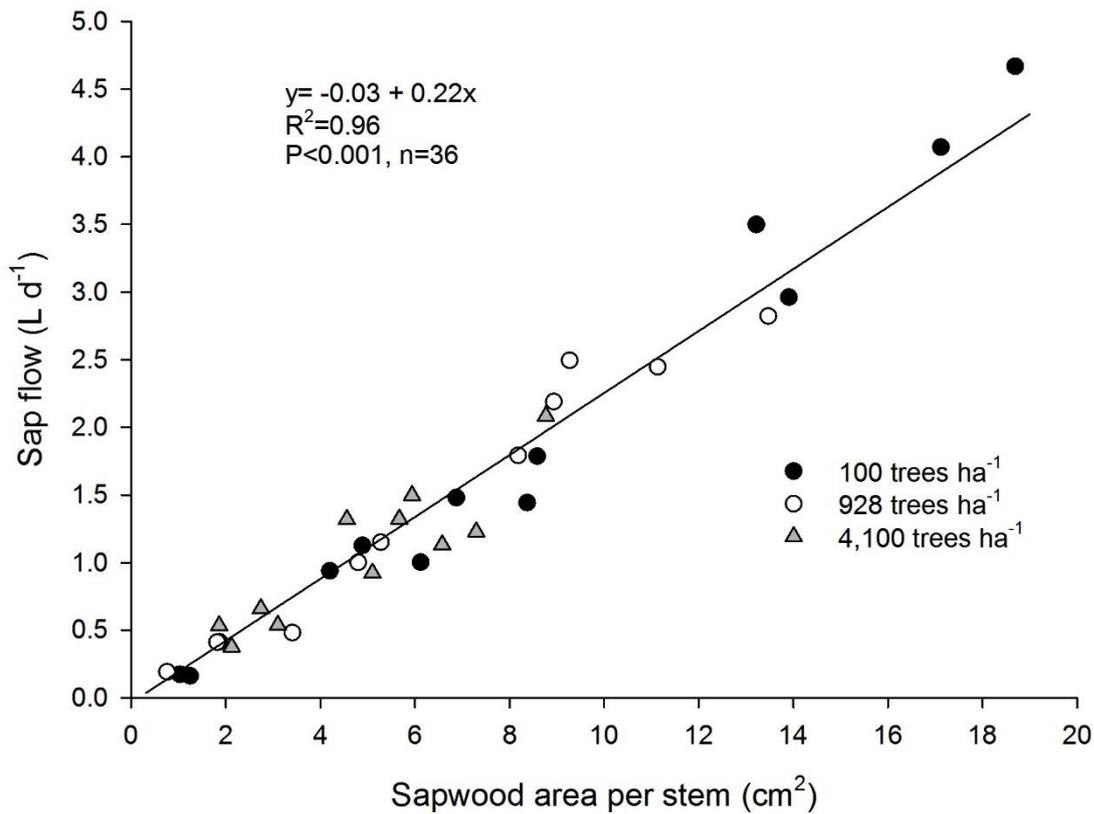


Fig. 4.8: Relationship between sapwood per stem (cm^2) and daily sap flow per stem (L day^{-1}) measured at three different densities. Daily sap flow per stem was corrected by VDP.

Tree transpiration varied according to density of leucaena (Table 4.3). At low density, leucaena had a greater number of stems per tree, stem diameter and sapwood area, resulting in higher transpiration rates per tree ($14.8 \text{ L tree}^{-1} \text{ day}^{-1}$ at $100 \text{ trees ha}^{-1}$ compared to $1.6 \text{ L tree}^{-1} \text{ day}^{-1}$ at $4,100 \text{ trees ha}^{-1}$).

Table 4.3: Summary of tree characteristics and average leucaena transpiration ($\text{L day}^{-1} \text{ tree}^{-1}$) measured at three different densities in a Nelder fan experiment in March 2015.

Density (trees ha^{-1})	Range of N° of Stems tree^{-1}	Average stem diameter (cm)	Average sapwood area per tree ($\text{cm}^2 \text{ tree}^{-1}$)	Average transpiration per tree (L day^{-1})
100	4-11	5.27 ± 0.25	60.3 ± 2.09	14.8 ± 2.5
928	3-9	4.05 ± 0.25	29.7 ± 2.21	7.2 ± 0.5
4,100	1-5	3.57 ± 0.35	3.8 ± 0.76	1.6 ± 0.2

4.4 Discussion

4.4.1 Effect of defoliation on water use of leucaena (Experiment 1)

Leucaena plants had different water uptake according to intensity of defoliation and time of recovery. At high levels of defoliation, water uptake followed the sigmoidal patterns suggested by Stur *et al.* (1994) for regrowth. There was a lag phase where plants used negligible water as all photosynthetic tissues were removed. When plants had regenerated new leaves and shoots, a second phase started after ~ 10 days and water uptake increased markedly. These responses were not evident at lower defoliation intensities which continued high levels of water use. It was also observed that low defoliation intensity may have increased

water uptake of leucaena plants probably due to stimulation of new leaf and shoot growth compared to leucaena plants without defoliation.

Similar responses of stimulation on leaf and shoot were reported by Teague and Walker (1988) working with different intensities of defoliation of the tree legume *Acacia karroo* by goats. They reported considerable stimulation of leaf and shoot growth compared to non-defoliated plants, and this response differed according to the intensity and phenophase¹ of defoliation. They also found that *Acacia karroo* plants were sensitive to defoliation when carbohydrate reserve levels were low, but were tolerant of defoliation when reserves were high. In the experiment, carbohydrate reserves was not measured but it was hypothesised that levels of reserves were adequate to high as the plants had not previously experienced defoliation.

Reserves of carbohydrates in woody species (i.e. starch, soluble sugar) have been reported to be responsible for new growth after defoliation. There is evidence that plants mobilize their reserves to rebuild photosynthetic tissues after defoliation, cutting or seasonal loss of foliage (Latt *et al.* 2000). Research on carbohydrates in *Leucaena leucocephala* and *Gliricidia sepium* showed that level of reserves was affected by post-cutting biomass and that frequent cutting progressively decreased concentrations of starch and reserves of carbohydrates (Latt *et al.* 2000).

Therefore, it suggested that in the experiment, the increases of water uptake observed after defoliation were a result of increased photosynthetic tissues due to mobilization of carbohydrate reserves from root or stem. However, this response may differ with successive defoliations; there is evidence that when trees are cut often, carbohydrate reserves are progressively depleted unless there is sufficient time for replenishment of carbohydrate reserves and with leucaena this process could take four to six weeks (Guevarra, 1978; Latt *et al.* 2000).

Regarding the relationship between remaining leaves and water uptake, leaf area per tree was an excellent predictor of water use of leucaena. This result was similar to that reported for other trees species, where parameters such as LAI and leaf area were the best predictors of transpiration as they are a measure of the evaporative surface area (Running and Coughlan, 1988; Santiago *et al.*, 2000). The “pipe model” theory (Shinozaki *et al.* 1964) suggested that branches and stems can be considered as pipes which are related to leaves that they support. It was hypothesised that leaf area per tree would be a good parameter to be used for modelling of water use of leucaena due to its close relationship with water use of the whole tree. It is also relatively easy to estimate using allometric equations based on basal diameter or cross-sectional area of sapwood (Fownes and Harrington, 1990).

4.4.2 Comparison between twinpot systems and sap flow meter (Experiment 2)

Our results suggested little difference between twinpot system and SFM for measurement of soil water uptake of leucaena plants, although, SFM may have slightly underestimated total water uptake. The twinpot system is a direct measure of water uptake and therefore whole-plant transpiration (Hunter *et al.* 2012). Sap flow meters are an indirect measure of sap flow rate (transpiration), which relies on measurement of changes in temperature of the xylem induced by an external addition heat pulse (Smith and Allen, 1996; McCulloh *et al.* 2007). No other evaluation of different approaches to measurement transpiration of leucaena plants has been sighted. However, a comparison of daily water use estimated by sap flow meter and gravimetric measurement of water use using pots (378 L plastic container) was reported for two tropical tree species *Pseudobombax septenatum* and *Calophyllum longifolium* (McCulloh *et al.* 2007). They found that a Granier-style heat dissipation sensor slightly underestimated (<3%) total daily water use

In another evaluation carried out in Southern Queensland, Australia with *Harpullia pendula*, a native subtropical and tropical tree, sap flow meters over-estimated transpiration rate by ~11% (Uddin, 2014). In terms of convenience, both systems have advantages and disadvantages. For twinpot systems, their main advantages (Hunter *et al.* 2014) are: accurate measurement of water use, low use of materials and labour,

¹ A *phenophase* is a distinct event in the annual life cycle of a plant or animal in relation to changes in seasons and climate. In plants, examples of these observable events include budburst, first flower, first ripe fruit, and color change.

easy to build, and it is possible to impose a range of treatments (e.g. water stress, fertilization). It can be also used from seedlings to relatively big trees. However, its main disadvantages are that automatization is limited which increases their cost and they cannot measure water use of trees growing in the field. The advantages of sap flow meters are well known (Smith and Allen, 1996; Vandegehuchte and Steppe, 2013; Steppe *et al.* 2015). They are relatively easy to use, accurate, portable, data loggers allow frequent sampling intervals and they can be used for measurement of stem, branches or trunks. Disadvantages are their relatively high cost per unit and measurement cannot be done on small stems (<10 mm diameter).

4.4.3 Transpiration of leucaena plants in the field (Experiment 3)

There is limited information in the literature about how much water is transpired by leucaena. Nyadzi *et al.* (2002) studied water use of three legume-trees: *Acacia crassicarpa*, *Leucaena pallida* and *Senna siamea* in an agroforestry system in Western Tanzania. They found that mean daily transpiration ranged 0.3 to 1.7 L day⁻¹ for all species and was largely related to stem diameters, size of canopy and soil moisture available. Trees of *L. pallida* that had small stems (<6 cm) and crown diameters (<3.5 m) transpired 0.3- 0.5 L day⁻¹. In glasshouse conditions, mean transpiration rates of leucaena were 3.3 L day⁻¹ and 2.2 L day⁻¹ for experiment 1 and 2 respectively. However, for leucaena growing under field conditions, transpiration ranged from 1.6 to 14.8 L day⁻¹. Those high values of transpiration of leucaena were similar to 12 L d⁻¹ measured in *Eucalyptus cloeziana* (11 cm diameter at breast height, DBH) and *E. pilularis* (12.5 cm DBH) in northern New South Wales Australia (Alcorn *et al.* 2013). They also reported maximum daily transpiration in both species of 18 L day⁻¹.

4.5 Conclusions

Low intensities of defoliation (25% leaves removed) may stimulate water uptake due to a positive response of increasing growth of new leaves and shoots. After 32 days, plants that experienced 75% defoliation, had similar rates of water uptake as the control treatment of no defoliation. When all leaves were removed or trees were cut leaving bare stems, water uptake of leucaena remained lower until trees developed new leaves and shoots. The strong relationship between leaf area and water uptake of leucaena should be considered as parameter in modelling and should be tested under field conditions.

Under field conditions, transpiration of leucaena varied according to plant density as higher rates of transpiration were measured in larger trees grown at low density.

Finally, leucaena transpiration rate can be accurately and reliably measured. using either twinpot systems or sap flow meter.

5 Above-ground interactions: The effect of tree density on competition between *Leucaena leucocephala* and *Chloris gayana* using a Nelder fan design

5.1 Introduction

Leucaena [*Leucaena leucocephala* (Lam.) de Wit ssp. *glabrata* (Rose) Zarate] is a multipurpose forage tree legume widely used for ruminant feeding in subtropical and tropical regions (Shelton and Brewbaker, 1994). In Queensland Australia, the leucaena-grass hedgerow silvopastoral system is the most productive, profitable and sustainable tropical pasture for beef cattle production (Shelton and Dalzell, 2007; Bowen *et al.* 2016). The major advantage of leucaena-grass pasture is superior nutritive characteristics (Shelton and Dalzell, 2007) compared to tropical grass pastures e.g.: Buffel grass (*Pennisetum ciliare* L.), Gatton panic (*Panicum maximum* Jacq.) and Rhodes grass (*Chloris gayana* Kunth). However, respective roles of leucaena and companion grass and therefore the most appropriate balance of species are continually debated. Worldwide, there is no consistency concerning the most appropriate tree densities and planting configurations for leucaena and grass forage systems. The significance of planting arrangement on the proportion of leucaena and grass on offer to grazing ruminants is poorly understood. In Queensland, leucaena densities range between 1,000 and 8,000 trees ha⁻¹ depending on planting configuration which varies with hedgerow spacing, density of plants within the hedgerows and whether single or twin hedgerows are planted (twin hedgerows are typically spaced 0.5-1 m apart). Hedgerows can be spaced from 4 to 15 m apart (Radrizzani *et al.* 2010). Elsewhere in the world, planting density is often much higher. For example, leucaena planted at 10,000 trees ha⁻¹ is intercropped with improved tropical grass pasture for direct grazing by livestock in Colombia and Mexico (Murgueitio *et al.* 2011).

In agroforestry systems, the degree of complementary or competitive interactions between species will depend on the ability of component species to capture resources and to use them effectively (Ong and Leakey, 1999). Competition occurs when overlapping plants reduce one or more of the growth resources to the point where the growth, reproductive or survival performance, of at least some plants, are negatively affected (Harper, 1990). When species are grown in monoculture, *intraspecific* competition occurs. When mixtures of species are grown together *interspecific* competition for resources occurs. Mixed species may have different resource requirements (light, water or nutrients) leading to complementary use of resources (Vandermeer, 1989; Forrester *et al.* 2006). Facilitation processes is another outcome and occurs when one species positively affects another in mixed plantings (Vandermeer, 1989).

Above-ground biomass productivity of leucaena-grass systems depends upon soil fertility, plant available soil water, species growth and rooting habits, cultivars used and climatic conditions. Tree density and row spacing also play a fundamental role in the intensity of leucaena-grass competition and the relative yield of the system components. In agroforestry systems, trees usually are the dominant competitor for light, while both trees and grass will compete for soil water and mineral nutrients. Few studies have focused on *intra* and *interspecific* competition within leucaena-grass systems.

The aim of this study was to determine *intra* and *interspecific* competition and complementarity levels of the effect of leucaena density on above-ground biomass of leucaena and grass in a mixed sward. Our hypotheses were: (a) Rhodes grass competition will greatly reduce above-ground biomass of leucaena when leucaena is grown at low density; (b) leucaena competition will greatly reduce Rhodes grass above-ground biomass when leucaena is grown at high tree density; and (c) that a Nelder fan experimental design will elucidate *intra* and *interspecific* competition effects on above-ground biomass of a leucaena and Rhodes grass sward.

5.2 Material and methods

5.2.1 Experiment site

The experiment was conducted at the University of Queensland Gatton Campus, Gatton, Queensland, Australia (27.54°S, 152.34°E). The soil was an alluvial Lockyer prairie soil (USDA Soil Taxonomy Fluventic haplustoll) (Isbell, 1996). Detailed soil physical and chemical characteristics were described by Powell (1982).

Briefly, soil texture was 5% coarse sand, 20% fine sand, 22% silt and 53% clay, soil pH ~ 6.7 , EC (0.15-1 m depth) 0.377 dS m^{-1} . The climate is subtropical, with average rainfall of 798 mm per annum and average maximum and minimum temperatures of 31.6 and 19.3°C in the hottest month (January) and 20.7 and 6.2°C in the coolest month (July). A weather station monitored daily solar radiation, temperature, humidity, wind speed and precipitation during the experimental period. Irrigation was applied via solid set sprinklers until November 2014. Daily average temperature and rainfall data are presented in Fig. 5.1; the trial area received 1,454 mm of rainfall from May 2014 to May 2016.

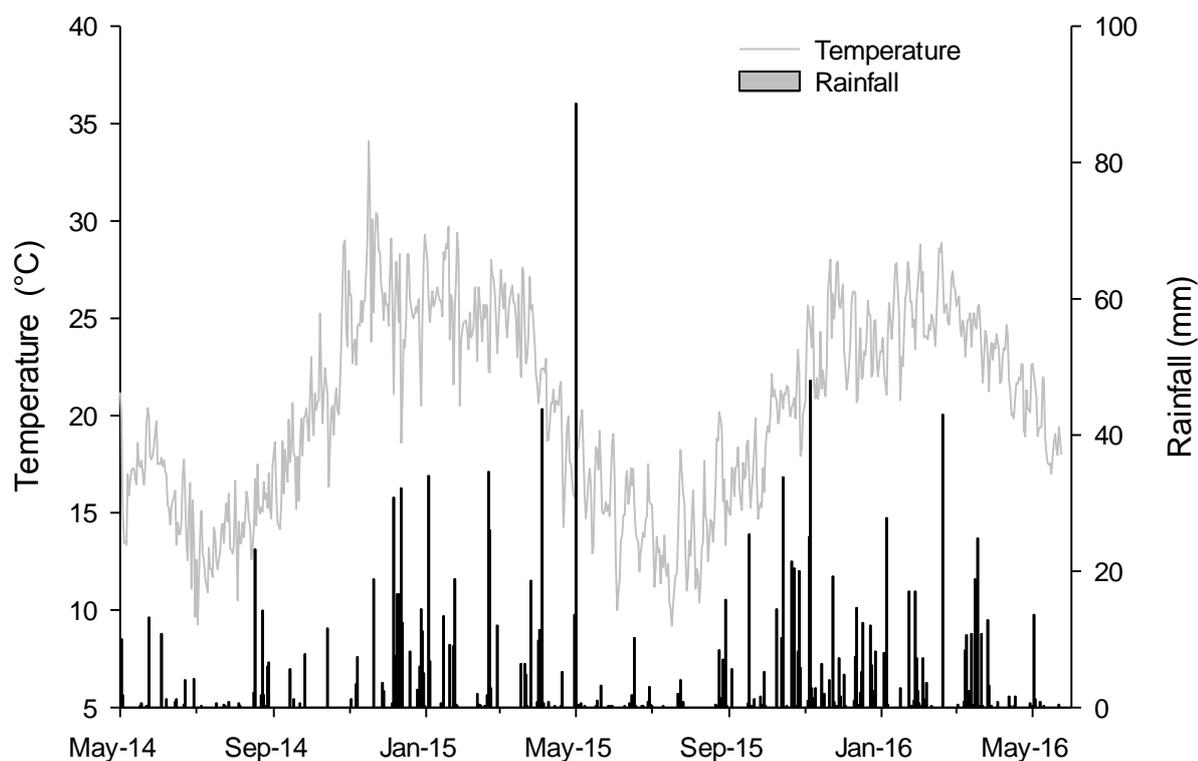


Fig. 5.1: Daily average temperature (°C) and rainfall events measured from May 2014 to May 2016.

5.2.2 Experimental design

Prior to planting leucaena, fertilizer was applied to the entire plot at a rate of $30.2 \text{ kg N ha}^{-1}$, 8.8 kg P ha^{-1} , 23 kg K ha^{-1} and $27.2 \text{ kg S ha}^{-1}$. Leucaena cv. Tarramba was planted using a Nelder fan design (Nelder, 1962) on 27 November 2013. Twelve concentric rings of trees with radii (spoke length) of 0.6, 0.9, 1.3, 1.9, 2.8, 4.0, 5.9, 8.5, 12.3, 17.9, 25.9 and 37.6 m were planted. Each ring contained 16 trees planted equidistant around the circumference, giving a range of tree densities from 100 to 80,000 trees ha^{-1} (Table 5.1). In this design, the rate of change along planting spokes (α), has been set at 1.45 with an angle between spokes (Θ) of 22.47° (0.3992 radians) generating 16 spokes. The trial occupied an area of 0.47 ha with 192 leucaena trees (Plate 5.1). The outer and the innermost ring and spokes 1, 5, 9 and 13 were used as guard rows of trees. Rhodes grass cv. Finecut was sown at 5 kg ha^{-1} on 11 March 2014 in two quarters of the Nelder fan (see Plate 5.1).

Table 5.1: Radial length (spoke), ring tree spacing and associated tree density and potential growing space of the Nelder wheel experiment.

Radius	Radius length (m)	Tree density (trees ha ⁻¹)	Growing space (m ²)	Spacing within rings (m)	Spacing within spokes (m)
r_0^*	0.6	-----	-----	-----	-----
r_1	0.9	80,000	0.13	0.36	0.28
r_2	1.3	38,065	0.26	0.52	0.41
r_3	1.9	18,112	0.55	0.75	0.60
r_4	2.8	8,618	1.16	1.09	0.87
r_5	4.0	4,100	2.44	1.58	1.25
r_6	5.9	1,951	5.13	2.29	1.82
r_7	8.5	928	10.77	3.31	2.64
r_8	12.3	442	22.64	4.80	3.82
r_9	17.9	210	47.58	6.96	5.54
r_{10}	25.9	100	100.00	10.09	8.04
r_{11}^*	37.6	-----	-----	-----	-----

* Inner and outer rings were used as guard rows.

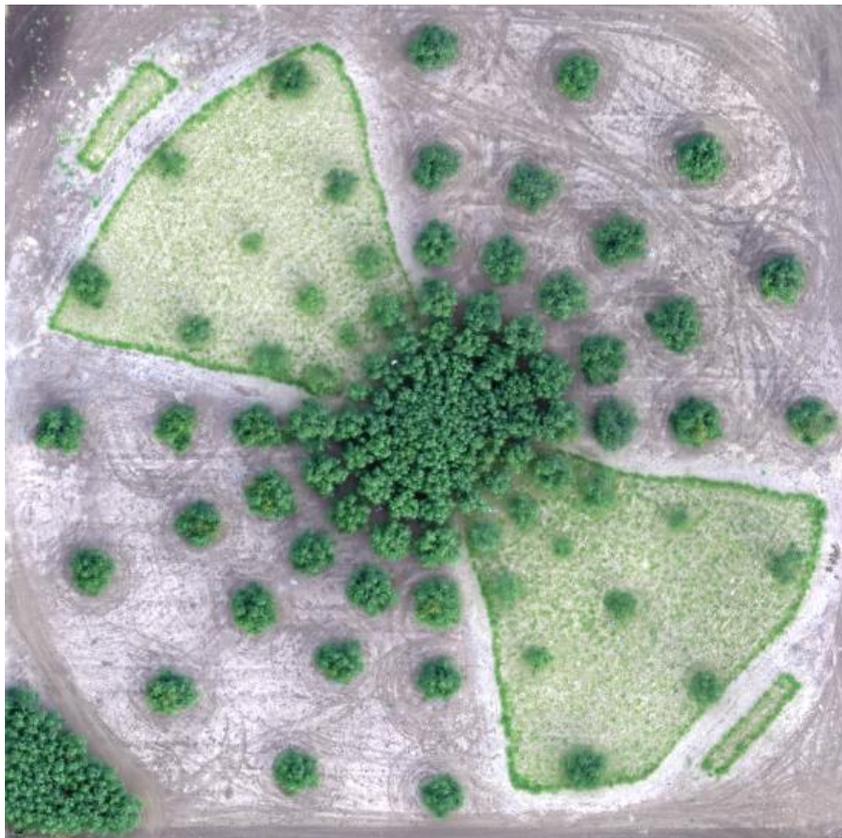


Plate 5.1: Aerial view of Nelder fan (25 August 2015) 636 days after establishment of leucaena trees and 532 days after establishment of Rhodes grass.

5.2.3 Measurement of leucaena yield, edible biomass yield, stem number, stem basal area, leaf area and leaf area index

The above-ground biomass of leucaena was measured at 6 harvests over 742 days (May 2014 to June 2016). The above-ground biomass of Rhodes grass was measured at 7 harvests over 721 days (June 2014 to June 2016).

At each harvest, 80 trees were sampled, corresponding to 8 trees per density (4 trees growing with Rhodes grass and 4 without grass competition). The leucaena was harvested to a height of 1 m and the basal diameter of all first degree regrowth stems cut (> 1 mm diameter) and measured with digital callipers. Simultaneously, for calibration purposes, the basal diameter of several stems per tree at a range of densities (34-45 stems) was measured and total biomass recorded. Branches were then separated into edible biomass (leaves and green stem <5 mm diameter) and non-edible (woody stems >5 mm diameter). At the same time, leaf area (cm²) was measured using a portable leaf area meter Li- 3000C (Li-Cor Lincoln, Nebraska, USA). Subsequently, fresh sub-samples were oven-dried at 65°C for 72 hours to determine dry matter content.

Using a regression relationship between total biomass (g DM stem⁻¹) and leaf area (cm² stem⁻¹) with the cross-sectional area of the cut stems (cm²) and number and basal diameter of stems per tree, the individual biomass per tree (kg DM tree⁻¹) and leaf area per tree (cm² tree⁻¹) were estimated. This methodology was used at each harvest. The regression coefficients (R²) linking total biomass with cross sectional area of the stem were 0.91, 0.99, 0.99, 0.98, 0.90 and 0.95 (all $P < 0.001$) corresponding to harvests on 10/10/14, 9/12/14, 21/01/15, 12/10/15, 2/02/16 and 24/05/16 respectively. Total and edible biomass per area (kg DM ha⁻¹ year⁻¹) was estimated from leucaena density and the individual biomass per tree using the data from harvests 1 to 4 for period 1 (2014-2015-year 1) and harvests 5 and 6 for the period 2 (2015-2016-year 2).

The regression coefficients (R²) for leaf area were 0.98, 0.99, 0.95, 0.98, 0.89 and 0.88 (all $P < 0.01$) corresponding to the aforementioned harvests. Leaf Area Index (LAI) was calculated using the leaf area per tree and tree density.

5.2.4 Measurement of Rhodes grass yield

The above-ground biomass of Rhodes grass (edible biomass) 0.1 m above the soil surface was estimated using BOTANAL sampling procedures (Tothill *et al.* 1978) comprising a visual scoring system to estimate grass yield and calibration equations to convert the visual scores to biomass yield. At each harvest, 148 quadrants (0.4 x 0.4 m) were scored in each grass replication. Additionally, pasture samples (12–15) were harvested for calibration purposes; fresh biomass was measured and sub-samples oven-dried at 65°C for 72 hours to determine dry matter content. Regression calibration equations linking biomass to visual yield score had R² values of 0.92, 0.97, 0.99, 0.94, 0.98, 0.94 and 0.98 (all $P < 0.001$) for harvests taken on 7/10/14, 19/11/14, 20/01/15, 24/06/2015, 20/10/2015, 2/02/16 and 24/05/16 respectively.

5.2.5 Nutrient status of leucaena and Rhodes grass

Leaf samples of leucaena and Rhodes grass were collected in December of 2014 and 2015. For leucaena, youngest fully expanded leaves (YFEL) were selected as described by Radrizzani *et al.* (2011a) at densities of 100, 442, 928, 1951, 4100, 8618, 18,112 and 80,000 trees ha⁻¹, with and without grass competition. For Rhodes grass, shoot samples that included leaf and stem were harvested at 0.1 m above ground at densities of 100, 928, 1951, 4100 and 8618 trees ha⁻¹. Plant tissue samples were oven-dried at 65°C for 72 hours, ground to pass a 1 mm sieve and analysed for nitrogen (N) by combustion analysis using a TruSpec CHN analyser (LECO Australia Pty. Ltd., NSW, Australia). Nitric perchloric acid digestion followed by analysis in inductively coupled plasma optical emission spectrometer (ICP-OES) (Vista Pro, Varian Inc., Australia) determined the concentrations of phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn).

5.2.6 Measurement of photosynthetic photon flux density (PPFD)

PPFD readings (μmol m⁻² seg⁻¹) were used to measure the extent of shading of Rhodes grass by the taller leucaena. Readings were taken between spokes immediately above the grass canopy (0.15 m from the soil surface) on a sunny day between 11:00 to 13:00 at 20 (22/02/16), 37 (10/03/16), 50 (23/03/16) and 84 (26/04/16) days after harvest of leucaena trees (2/02/2016) using a lineal ceptometer (AccuPAR LP-80, Decagon Devices Inc, Pullman, Washington, USA). At very high densities, where the grass was not present, the measurements were made 0.15 m from the soil surface. The percentage of available PPFD was calculated

as the relationship between the average PPFD for each tree density and the average PPFD without trees (full sun exposure).

5.2.7 Data analysis

Accumulated total above-ground biomass of leucaena ($\text{kg DM tree}^{-1} \text{ year}^{-1}$ and $\text{kg DM ha}^{-1} \text{ year}^{-1}$), edible above-ground biomass ($\text{kg DM ha}^{-1} \text{ year}^{-1}$) and edible Rhodes grass above-ground biomass ($\text{kg DM ha}^{-1} \text{ year}^{-1}$), individual leaf area ($\text{m}^2 \text{ tree}^{-1}$), LAI and number of stems per tree were plotted against leucaena density (trees ha^{-1}) and presented as scatter plots. Following the methodology of Ritchie (1997), tree density was \log_{10} transformed and the data subjected to non-linear and linear regression analysis. Non-linear regressions were developed for: a) accumulated individual tree above-ground biomass of leucaena; b) accumulated edible above-ground biomass for leucaena and Rhodes grass; c) leucaena and Rhodes grass percentage of combined edible biomass; and d) percentage of PPFD transmission. Linear and quadratic regressions using Minitab (Version 16.2.4, MiniTab Inc, State Collage, Pennsylvania, USA) were developed for total stem cross-sectional area, number of stems per tree, leaf area, LAI, canopy light interception and tissue nutrient concentration against leucaena tree density ($\log_{10} \text{ trees ha}^{-1}$).

5.3 Results

5.3.1 Leucaena total biomass yield per tree

Maximum average cumulative above-ground total biomass yield per tree was produced at 100 trees ha^{-1} without grass competition in both years; yields were 23.7 and 54.2 kg DM tree^{-1} in years 1 and 2 respectively. Leucaena plants growing with grass competition reached maximum individual tree total biomass yield at densities ranging from 100 to 928 tree ha^{-1} (average of 7.6 kg DM tree^{-1}). However, with further increments of density, total biomass yield per tree of leucaena was negatively correlated to \log_{10} of tree density ($R^2=0.99$). Tree total biomass yield at the highest density was 0.42 kg DM tree^{-1} in year 1 and 0.75 kg DM tree^{-1} in year 2, and grass competition did not affect tree yield at densities ≥ 4100 trees ha^{-1} (Fig. 5.2).

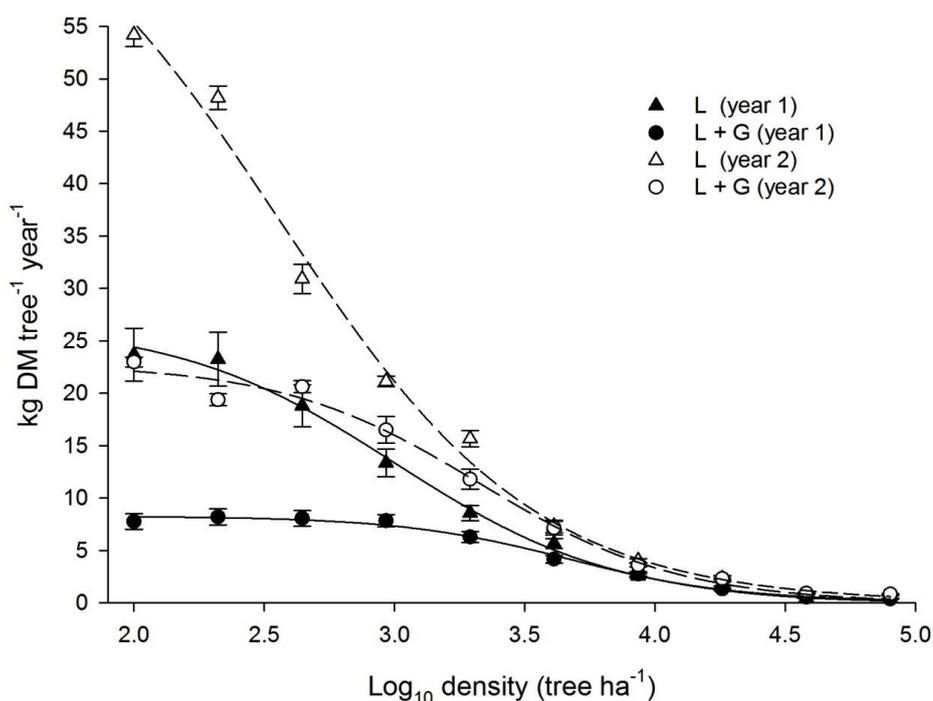


Fig. 5.2: Cumulative total biomass yield per leucaena tree ($\text{kg DM tree}^{-1} \text{ year}^{-1}$) grown with and without grass competition at 10 leucaena tree densities (trees ha^{-1}). Vertical bars indicate standard errors. Non-linear regression models were used: (\blacktriangle) $y=26.65/(1+\exp(-(x-3.00)/-0.42))$, $R^2=0.99$; (\bullet) $y=8.27/(1+\exp(-(x-3.67)/-0.33))$, $R^2=0.99$; (\circ) $y=73.32/(1+\exp(-(x-2.55)/-0.49))$, $R^2=0.99$ and (\triangle) $y=22.79/(1+\exp(-(x-3.32)/-0.38))$, $R^2=0.99$.

5.3.2 Leucaena total biomass yield per ha

Total biomass of leucaena was positively correlated to the \log_{10} density ($P < 0.001$). Maximum total biomass yield was reached, in both years, at 80,000 trees ha^{-1} regardless of grass competition; average yields were 32,924 and 60,214 kg DM ha^{-1} in years 1 and 2 respectively (Fig. 5.3). Minimum total biomass yield was reached, in both years, at 100 trees ha^{-1} with grass competition; yields were 780 and 2,298 kg DM ha^{-1} .

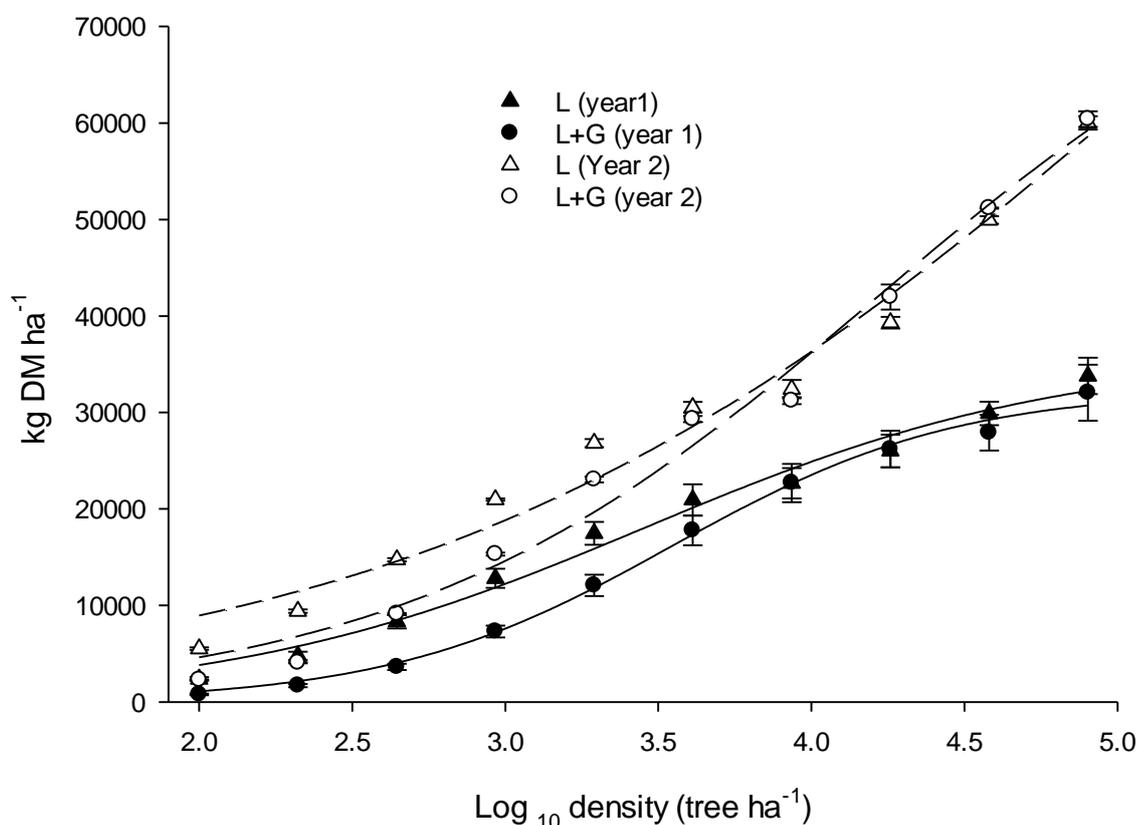


Fig. 5.3: Cumulative total biomass yield of leucaena (kg DM ha^{-1} year $^{-1}$) grown with and without grass competition at 10 leucaena tree densities (trees ha^{-1}). Vertical bars indicate standard errors. Non-linear regression models were used: (\blacktriangle) $y = 36139.1 / (1 + \exp(-(x - 3.45) / 0.68))$, $R^2 = 0.98$; (\bullet) $y = 32369.2 / (1 + \exp(-(x - 3.54) / 0.46))$, $R^2 = 0.99$; (\circ) $y = 131056.8 / (1 + \exp(-(x - 5.15) / 1.2))$, $R^2 = 0.97$ and (\triangle) $y = 83436.6 / (1 + \exp(-(x - 4.2) / 0.78))$, $R^2 = 0.98$.

5.3.3 Leucaena edible biomass yield per ha

The cumulative edible above-ground biomass per ha of leucaena was positively and significantly ($P < 0.001$) correlated to the \log_{10} density ($R^2 = 0.99$) regardless of grass competition, reaching 21,460 kg DM ha^{-1} in year 1 and 27,160 kg DM ha^{-1} in year 2 at the highest leucaena density of 80,000 tree ha^{-1} (Fig. 5.4a and 4.4b). In contrast, the cumulative edible above-ground biomass of Rhodes grass per ha was inversely correlated with the \log_{10} of tree density ($R^2 = 0.97$ and 0.94 in years 1 and 2 respectively) (Fig. 5.4a and 5.4b). Yield of Rhodes grass at the lowest tree density was 12,183 kg DM ha^{-1} in year 1 and 6,543 kg DM ha^{-1} in year 2. With increments of leucaena tree density, grass yield declined to 1,420 and 310 kg DM ha^{-1} at 8,618 trees ha^{-1} in years 1 and 2 respectively. At higher leucaena tree densities, the cumulative yield of Rhodes grass was close to zero.

The combined edible above-ground biomass of the leucaena-grass pasture is shown in Fig. 5.4a and 5.4b. Incorporating Rhodes grass into the leucaena trees increased edible biomass production at tree densities $\leq 8,618$ trees ha^{-1} . In year 1, Rhodes grass contributed most of the total yield at low density but made no significant contribution at leucaena densities of $\geq 8,618$ trees ha^{-1} . In year 2, the contribution of Rhodes grass to total yield was much lower.

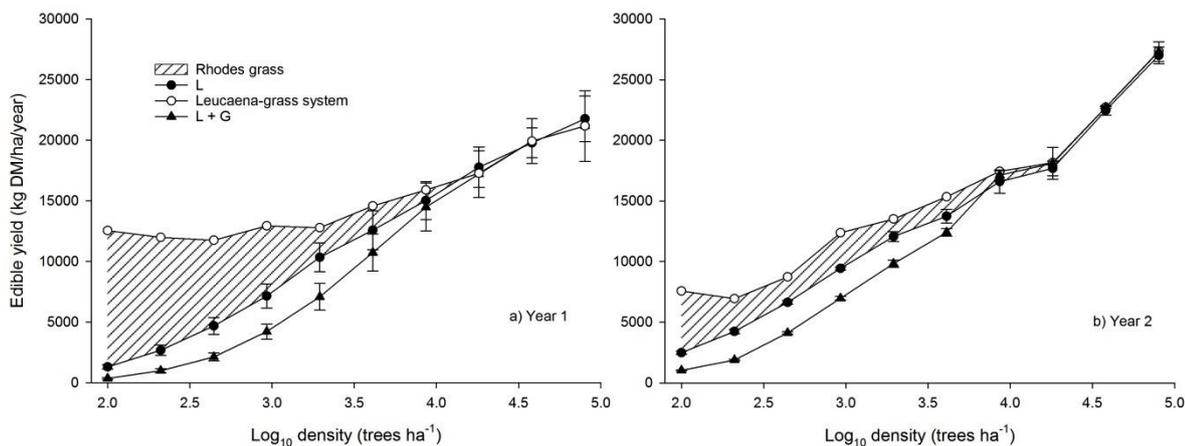


Fig. 5.4: Effect of leucaena tree density on cumulative edible above-ground biomass (kg DM ha⁻¹ year⁻¹) of leucaena growing with (▲, L+G) and without grass competition (●, L) above-ground biomass of combined leucaena-grass pasture (○, Leucaena-grass systems) and above-ground biomass of Rhodes grass (area filled, Rhodes grass) at year 1 (a) and year 2 (b). Vertical bars indicate standard errors.

5.3.4 Percent contribution of leucaena and Rhodes grass to combined edible biomass yield

The percentage contribution of leucaena and Rhodes grass to the combined edible biomass of the leucaena-grass system averaged across years 1 and 2 varied with tree density (Fig. 5.5). Leucaena composition, averaged for year 1 and 2, was 8.2, 64 and 94% of total biomass at tree densities of 100, 1,951 and 8,618 trees ha⁻¹ respectively.

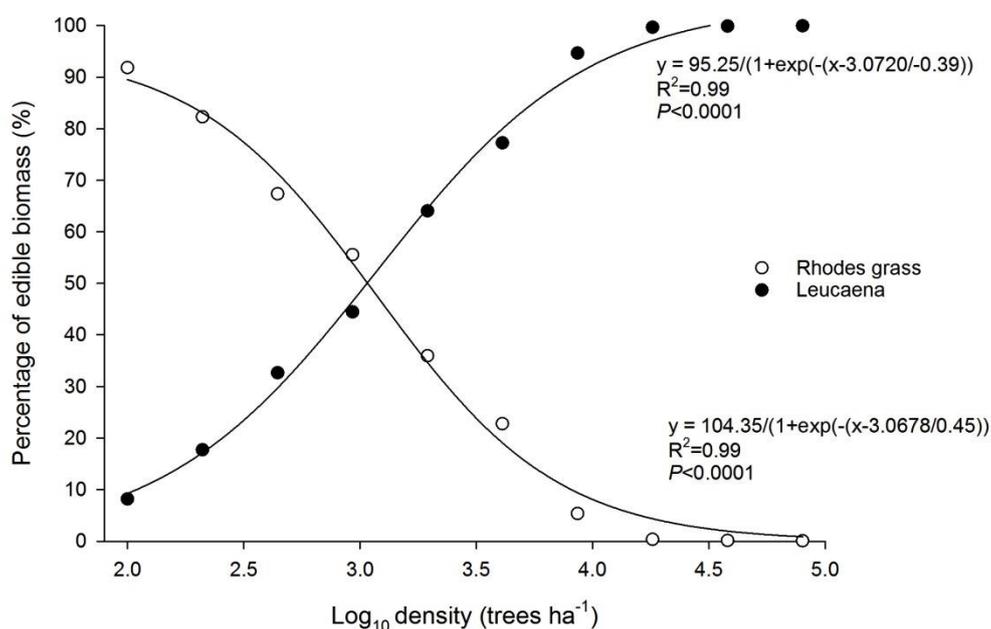


Fig. 5.5: Contribution (%) of leucaena (●) and Rhodes grass (○) to combined total edible biomass averaged for years 1 and 2 at different leucaena tree densities.

5.3.5 Canopy light interception

PPFD available to Rhodes grass declined with leucaena tree density and number of days of regrowth following harvest of the leucaena trees (Fig. 5.6). For instance, after 20 days of regrowth, light transmission to Rhodes grass was 99% at 100 trees ha⁻¹ and 61% at 80,000 trees ha⁻¹. However, after 84 days of regrowth, light transmission decreased to 84% at 100 trees ha⁻¹ and 10% at 80,000 trees ha⁻¹.

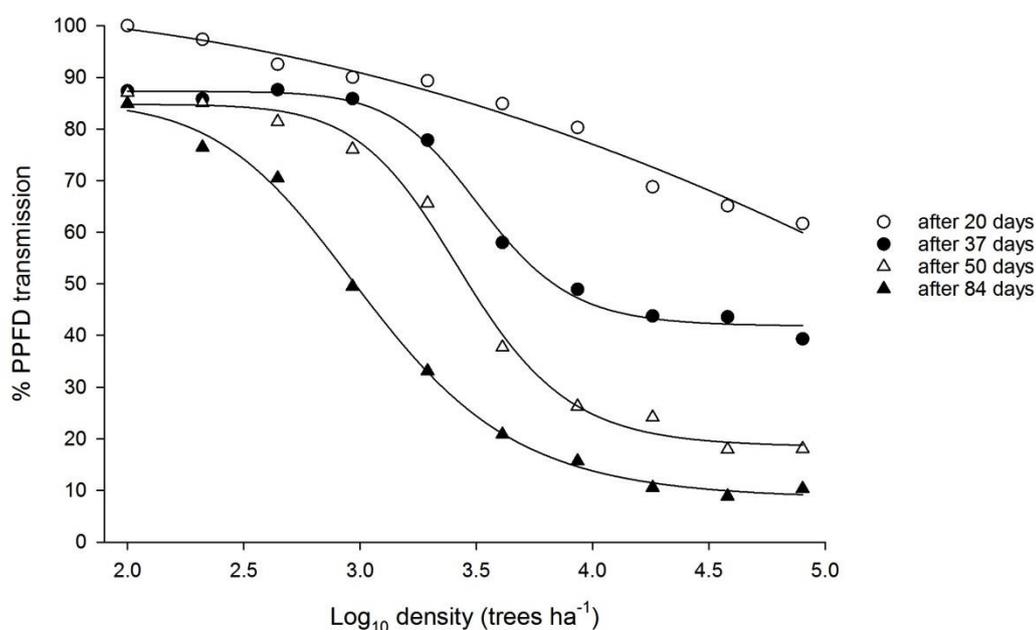


Fig. 5.6: Average PPFD (%) available for Rhodes grass at different leucaena tree densities measured 20, 37, 50 and 84 days after harvest of leucaena. Models used were highly significant $P < 0.001$: (○) $y = 100 + 5.0480x - 2.6985x^2$, $R^2 = 0.98$; (●) $y = 41.8 + (87.3 - 41.8) / (1 + (x/3.52)^{17.95})$, $R^2 = 0.99$; (△) $y = 18.43 + (84.7 - 18.43) / (1 + (x/3.44)^{15.03})$, $R^2 = 0.99$ and (▲) $y = 8.27 + (85.18 - 8.27) / (1 + (x/3.03)^{9.25})$, $R^2 = 0.99$.

5.3.6 Plant nutrient status

There was no significant effect of leucaena tree density and grass competition on nutrient concentrations in youngest fully expanded leaves (YFEL) of leucaena. Tissue concentrations were averaged across all treatments (Table 5.2). All nutrients except for P and Zn, which were marginal, were in adequate concentrations to maintain high levels of plant growth (Radrizzani *et al.* 2011a).

Shoot concentrations of all nutrients in Rhodes grass shoots were above critical levels (Reuter and Robinson, 1996) except for N at low tree densities ($< 4,100$ trees ha^{-1}) (Table 5.3). Concentrations of N, P, S, K, Mg, Mn and Cu shoots increased ($P < 0.001$) in with leucaena tree density (Table 5.3).

Table 5.2: Average concentrations of N, P, K, Ca, Mg and Cu in YFEL of leucaena. Data from all tree densities in years 1 and 2 were pooled.

Nutrient concentration	Mean \pm S.E	Critical concentration
N (% DM)	4.9 \pm 0.1	adequate
P (%DM)	0.17 \pm 0.01	deficient
K (%DM)	1.1 \pm 0.02	adequate
S (%DM)	0.3 \pm 0.01	adequate
Ca (%DM)	0.9 \pm 0.05	adequate
Mg (%DM)	0.4 \pm 0.01	adequate
Cu (mg kg^{-1})	5.1 \pm 0.3	adequate
Zn (mg kg^{-1})	9.4 \pm 0.5	marginal

Table 5.3: Average concentrations of N, P, K, S, Mg, Mn and Cu in Rhodes grass shoots averaged for years 1 and 2 and their relationship with leucaena density. Values in brackets correspond to \log_{10} (tree density).

Nutrient concentration	Tree density (trees ha ⁻¹)					Regression analysis	Critical value*
	100 (2.0)	928 (3.0)	1,951 (3.3)	4,100 (3.6)	8,618 (3.9)		
N (%DM)	0.9	1.0	1.2	1.6	2.0	$y = 0.484x^2 - 2.320x + 3.63$ $R^2 = 0.99$	1.3
P (%DM)	0.20	0.25	0.29	0.28	0.27	$y = -0.029x^2 + 0.213x - 0.10$ $R^2 = 0.93$	0.19-0.25
K (%DM)	0.59	0.60	0.74	0.85	1.01	$y = 0.191x^2 - 0.913x + 1.64$ $R^2 = 0.98$	0.6-0.8
S (%DM)	0.10	0.11	0.14	0.14	0.17	$y = 0.018x^2 - 0.078x + 0.1$ $R^2 = 0.98$	0.07-0.12
Mg (%DM)	0.2	0.23	0.27	0.36	0.41	$y = 0.080x^2 - 0.363x + 0.60$ $R^2 = 0.98$	n/a
Mn (mg/kg)	16.7	20.4	26.1	33.4	45.4	$y = 11.50x^2 - 53.73x + 78.23$ $R^2 = 0.99$	n/a
Cu (mg/kg)	2.5	2.6	3.6	4.8	6.3	$y = 1.847x^2 - 8.939x + 12.93$ $R^2 = 0.99$	n/a

* Critical values from Reuter and Robinson (1996)

5.3.7 Cross-sectional basal area, number of stems, edible biomass (%), leaf area and leaf area index

The average sum of cross-sectional basal area (BA) of first degree regrowth stems per tree, leaf area and leaf area index were all influenced by tree density, grass competition and year of study (Table 5.4).

Average BA per tree was negatively related ($P < 0.001$) to \log_{10} of leucaena tree density and was much greater in year 2 compared to year 1, especially at low tree densities. Grass competition greatly reduced BA at low tree densities but had no effect at higher tree densities ($> 4,100$ trees ha⁻¹).

Similarly, the number of stems per tree was negatively related ($P < 0.001$) to \log_{10} leucaena tree density. Highest number of stems (128 and 193 stems tree⁻¹) was registered in trees growing at the lowest density (100 trees ha⁻¹) without grass competition in years 1 and 2 respectively. At the highest leucaena tree density, the number of stems was 2 and 3 stems tree⁻¹ in years 1 and 2 respectively regardless of grass competition. The average percentage of total biomass of leucaena per tree that is edible reduced from year 1 (70%) to year 2 (58%) and was higher with Rhodes grass. There was no effect of leucaena tree density on percentage of edible biomass.

Individual leaf area per leucaena tree was negatively related ($P < 0.001$) to tree density and grass competition but was not influenced by year. Maximum average leaf area per tree of ~ 47 m² was measured without grass competition at 100 trees ha⁻¹ and this decreased to 17-20 m² tree⁻¹ with grass competition at the same tree density and then to 1-5 m² tree⁻¹ for densities ≥ 8618 trees ha⁻¹.

Leaf area index was positively related to \log_{10} leucaena density ($P < 0.001$). Differences due to grass competition were observed at low density (100 trees ha⁻¹) but reduced with each increment of density.

Table 5.4: Average total cross-sectional basal area per tree (BA) ($\text{cm}^2 \text{ tree}^{-1}$), number of first degree stems per tree, percentage of edible biomass per tree (%), leaf area per tree ($\text{m}^2 \text{ tree}^{-1}$) and leaf area index (LAI) of leucaena growing with and without grass competition, at four densities. Values are averages (\pm standard error) for all harvests for years: 1 and 2.

Parameter	Year 1		Year 2	
	L	L+G	L	L+G
BA ($\text{cm}^2 \text{ tree}^{-1}$)				
100 trees ha^{-1}	99 \pm 31	38 \pm 7	242 \pm 16	99 \pm 15
928 trees ha^{-1}	59 \pm 16	32 \pm 6	90 \pm 1	53 \pm 13
8,618 trees ha^{-1}	12 \pm 2	13 \pm 2	17 \pm 3	14 \pm 3
80,000 trees ha^{-1}	2 \pm 0.4	2 \pm 0.4	3 \pm 0.6	3 \pm 0.2
Significance	***	***	***	***
Stems (stems/tree)				
100 trees ha^{-1}	128 \pm 20	76 \pm 13	193 \pm 46	74 \pm 15
928 trees ha^{-1}	86 \pm 22	61 \pm 14	93 \pm 15	70 \pm 21
8,618 trees ha^{-1}	35 \pm 9	30 \pm 5	27 \pm 2	24 \pm 6
80,000 trees ha^{-1}	5 \pm 2	6 \pm 2	6 \pm 1	6 \pm 2
Significance	***	***	***	*
Edible biomass (%)				
100 trees ha^{-1}	64 \pm 10	73 \pm 9	50 \pm 1	57 \pm 5
928 trees ha^{-1}	65 \pm 10	69 \pm 9	55 \pm 1	61 \pm 12
8,618 trees ha^{-1}	72 \pm 9	72 \pm 11	61 \pm 9	66 \pm 12
80,000 trees ha^{-1}	74 \pm 10	75 \pm 9	56 \pm 4	62 \pm 4
Significance	*	ns	ns	ns
Leaf area ($\text{m}^2 \text{ tree}^{-1}$)				
100 trees ha^{-1}	47 \pm 19	17 \pm 7	46 \pm 7	21 \pm 2
928 trees ha^{-1}	24 \pm 9	17 \pm 6	18 \pm 4	13 \pm 3
8,618 trees ha^{-1}	4 \pm 1	5 \pm 2	3 \pm 1	3 \pm 1
80,000 trees ha^{-1}	1 \pm 0.1	1 \pm 0.1	1 \pm 0.1	1 \pm 0.1
Significance	***	**	***	***
LAI ($\text{m}^2 \text{ m}^{-2}$)				
100 trees ha^{-1}	0.4 \pm 0.2	0.2 \pm 0.1	0.5 \pm 0.1	0.2 \pm 0.1
928 trees ha^{-1}	1.9 \pm 0.8	1.6 \pm 0.6	1.6 \pm 0.3	1.2 \pm 0.2
8,618 trees ha^{-1}	3.4 \pm 1	4.5 \pm 1.6	2.9 \pm 1.1	2.8 \pm 0.6
80,000 trees ha^{-1}	5.6 \pm 1.9	5.7 \pm 2	4.9 \pm 1.9	4.1 \pm 0.5
Significance	***	***	***	***

Significance of regression relationships between \log_{10} tree density and each attribute at each harvest (*= $P<0.05$, **= $P<0.01$, ***= $P<0.001$, ns=non-significant).

5.4 Discussion

This Nelder fan experiment has demonstrated the significant effects of tree density and grass competition on intra- and interspecific competition in the leucaena-grass silvopastoral system. Limited complementarity was observed as there was no increase in biomass yield of the combined system. The outcomes of competition and complementarity of leucaena-grass pasture are now discussed including future research issues.

5.4.1 Intra and interspecific competition effects on leucaena growth

Increasing leucaena tree density resulted in intra and interspecific competition that impacted leucaena and Rhodes grass biomass production. The significance of each component varied with leucaena tree density. Intraspecific competition was most pronounced at high tree density and the interspecific competition acute at low tree density. When leucaena and grass were combined, the measured outcome (e.g. leaf area, edible biomass yield) was the net combination of interspecific and intraspecific interactions between the plant species (Forrester *et al.* 2006).

For leucaena at high tree densities, intraspecific competition reduced individual tree total biomass, leaf area and number of stems per tree. We hypothesized that this diminution of biomass yield was mainly due to competition for moisture and to a lesser extent competition for light and nutrients. The data on water use of leucaena, reported separately (Chapter 6), indicated lower soil moisture content at leucaena tree densities $>8,618$ trees ha^{-1} due to the greater water extraction ability of leucaena plants. Interspecific light competition was not a significant factor for leucaena due to its competitive canopy height advantage compared to Rhodes grass; however light was limiting from at medium to high tree densities due to intraspecific competition. This was reflected in the very high LAI values at these densities. Leucaena requires high levels of light intensity for maximum growth but does show moderate tolerance to shaded conditions (Benjamin *et al.* 1991). However, the study of the shade tolerance of 14 legumes (Wong *et al.* 1985) reported that leucaena yield, leaf area and amount of stem were strongly reduced by 66% shaded conditions. Thus at high densities and high LAI, shading at lower leaves would have reduced their growth and this was confirmed by observation of leaf shedding even at times of high soil moisture. In terms of plant tissue nutrient analysis, phosphorus was found to be at deficient levels (Radrizzani *et al.* 2011a) independent of leucaena density and grass competition, and indicated that the initial fertiliser application did not replace nutrients removed in leucaena biomass throughout the experiment.

At low tree densities (<928 trees ha^{-1}), the yield of individual leucaena trees growing with Rhodes grass was reduced by approximately 50-70% suggesting strong interspecific competition. It is hypothesized that Rhodes grass reduced leucaena growth mainly due to its greater ability to compete for plant available soil moisture (Chapter 6); light and plant nutrients (with exception of phosphorus) were not limiting at these tree densities. A parallel study carried out at the same site on the root distribution of leucaena and Rhodes grass reported greater abundance of fine grass roots (8-10 times) compared to leucaena in the top metre of the soil profile (Chapter 6). Usually, trees have greater lateral spread and deeper roots than grasses, and are thus able to exploit different soil strata. However, it was noted that when trees are grown together with grasses and crops, root architecture can be variable and can be superficial in depth and intermediate in lateral extent (van Noordwijk *et al.* 1995). Thus competition with other species can be high. In a study of leucaena-grass pasture systems, in Southern Queensland, Pachas *et al.* (2016) reported strong interspecific competition for water resources in the upper 1.5 m soil profile as leucaena roots were exploiting 75% soil moisture compared with 90% of Rhodes grass

5.4.2 Inter and intraspecific competition effects on Rhodes grass growth

As expected, density of leucaena trees was the principal factor affecting interspecific competition with Rhodes grass. Rhodes grass was mainly affected by light and lesser extent by water (Chapter 6).

Asymmetrical competition between trees and grasses is well known as trees can shade grasses but not vice versa (Forrester *et al.* 2006). In agroforestry experiments in South-eastern Queensland, Rhodes grass has shown a positive yield response under moderate shade, indicating a degree of shade tolerance (Dunn *et al.*

1994, Wilson, 1996). In the current experiment, Rhodes grass growth was negatively affected by shading from leucaena canopies to the point where growth was negligible at the highest densities. Longer intervals between cutting of leucaena greatly reduced light transmission to the companion grass. Considering a shade level ~50% as a threshold, cutting intervals or grazing intervals would need to be shorter at high leucaena densities if the aim was to reduce the level of light competition. To a lesser extent, it was observed that interspecific competition for water resources also increased with density of leucaena due to the increasing abundance of fine roots of leucaena and decreasing abundance of fine roots of Rhodes grass (Chapter 6). During the second year, shortage of nitrogen due to intraspecific competition caused a marked reduction in Rhodes grass yield growing at low leucaena density. Nutrient tissue analyses showed deficient levels of N. In the field, leaves were yellow in colour at low to medium leucaena tree densities. Rundown of N available to grass pastures sown on fertile soils is an important issue in Australia. Studies in central and southern Queensland (vertosol-alluvial soils) reported that grass yield declined due to rundown of nitrogen in the soil profile several years after establishment (Radrizzani *et al.* 2011b; Peck *et al.* 2011). In the current experiment, there would have been little transfer of biologically fixed N as all harvested leucaena was removed from the site. At higher leucaena densities, reduced grass growth due to competition for light would have reduced growth and N uptake with the result that tissue N concentrations were higher.

5.4.3 Complementarity and facilitation in leucaena-grass pasture systems

It was expected that complementarity, or facilitation, would lead to a greater yield of the two species combined compared to either alone (Ong *et al.* 1996). Usually, trees have greater lateral spread and deeper roots than grasses, and are thus able to exploit different soil strata leading to a degree of complementary in their use of soil resources (Schroth, 1999). In this experiment, leucaena-grass pasture demonstrated a small degree of complementarity and/or facilitation of resources; however, the positive outcomes were not reflected in improved productivity due to strong interspecific competition.

Some complementarity for water resources occurred as leucaena roots explored deeper soil profile than Rhodes grass (Chapter 6). Facilitation may have occurred due to nitrogen fixation by leucaena as demonstrated by higher grass N contents, as well as higher concentrations of other nutrients, with increment of leucaena densities. A possible hypothesis explaining the increasing nutrient concentrations observed in grass tissues was a shade effect. A number of authors have reported positive effects of shade on nutrient concentration levels in grasses (Eriksen and Whitney 1981; Wilson *et al.* 1990; Belsky 1992; Jackson and Ash 1998; Pachas *et al.* 2014). Shade conditions increase the availability of soil nitrogen due to enhanced soil mineralization (Wilson and Wild, 1990). Reduced grass yield under tree canopies may also result in less dilution of nutrients (Cameron *et al.* 1989) and better nutrient status under the canopy zone due to leaf fall and litter decomposition (Ludwing *et al.* 2001). Since animal grazing was not included during the experiment, N transfer via urine and faeces did not occur. Further studies on N fixation of leucaena and transfer pathways between different densities of leucaena and grass are required to provide a better understanding of N transfer.

5.4.4 Practical recommendations

There is a limitation to the extent that it is possible to extrapolate from plant densities in a Nelder fan experiment to the Queensland on-farm hedgerow system. Outcomes could be different according to different planting configurations (twin hedgerows), type and depth of soil, and species and vigour of grass competition.

In practical situations, wider rows of leucaena will compromise the performance of leucaena due to vigorous companion grasses competing for soil moisture. Narrow rows will increase the density of leucaena and consequently the yield and proportion of leucaena will be greater. However, if the rows are too narrow, the persistence of grasses will be compromised due to shade competition for light and water uptake by leucaena plants.

A highlight of this study was the great reduction of leucaena yield at low density due to grass competition (~ 50–70%). If the aim of the grazer is to increase the amount of leucaena available, options are to increase the

area of leucaena grass-pasture planted or to increase the density of the leucaena hedgerows allowing access to grass pastures adjacent to the leucaena plantation.

In others countries such as Mexico and Colombia, intensive silvopastoral systems (ISS) are promoted by government, research organizations and universities and are being adopted graziers. In ISS, leucaena is planted at high densities (over 10,000 trees ha⁻¹) and combined with improved grasses. High stocking rates and improved meat and milk production in these systems were achieved with considerable reduction of external inputs using rotational grazing and a permanent water supply for cattle (Murgueitio *et al.* 2011). In Indonesia, leucaena plant densities vary considerably depending on planting method including alley cropping, boundary plantings or high density plantations (2 x 2 m apart). The system differs from that used in Australia and Latin America as Asian farmers use cut-and-carry systems for animal feeding. Several studies carried out in village environments confirmed the importance of leucaena as a forage resource for ruminant feeding. For instance, in Sumbawa, Eastern Indonesia, the average daily gain of Bali Bulls was improved 60% compared to traditional feeding (Panjaitan *et al.* 2014)

Thought needs to be given to most appropriate balance of legume and grass. Low densities of leucaena lead to greater percentage of grass but sacrifice leucaena yield; whereas, high tree densities lead to leucaena dominance which is favoured in countries such as Mexico, Colombia and Indonesia In these examples, high density leucaena can double the amount of leucaena forage available, while grass or roughage can be provided from other paddocks or as a supplement.

5.5 Conclusions

The Nelder fan design enabled the evaluation of the effect of leucaena density on intra and interspecific competition between leucaena and Rhodes grass. In this experiment, combining leucaena and grass pasture led to a limited degree of complementarity and facilitation of resources as reflected by the marginal improvement in system productivity. There was strong intra and interspecific competition for light and water which was dominant over potential positive interactions resulting from improved grass nitrogen nutrition. Grass competition reduced leucaena yield (50-70%) at low tree densities (100 to 4,100 trees ha⁻¹). Meanwhile, trees growing at high densities reduced grass yield due to light interception. The percentage of combined edible biomass attributable to leucaena and Rhodes grass varied with the density of leucaena reaching 90% leucaena when tree density was >8,000 trees ha⁻¹. Parallel studies focusing on below-ground competition such as root architecture and patterns of soil water extraction will provide a better understanding of grass competitive interactions between leucaena and grass in silvopastoral systems. Thought needs to be given to most appropriate balance of legume and grass in pasture systems. Moderate densities of leucaena led to a desirable balance of grass/legume (60/40%) but sacrificed leucaena yield, whereas, high tree densities led to high leucaena availability which works well in countries such as Mexico, Colombia and Indonesia. High density leucaena can double the amount of leucaena forage available which is inefficient use of protein unless grass/roughage can be provided from other paddocks or as a supplement.

6 Below-ground interactions. The effect of tree density on root distribution and water use of *Leucaena leucocephala* and *Chloris gayana*

6.1 Introduction

Leucaena [*Leucaena leucocephala* (Lam.) de Wit ssp. *glabrata* (Rose) Zarate] hedgerows combined with a compatible grass, is one of the most sustainable pasture systems for beef production in subtropical and tropical Australia. *Leucaena*-grass pasture increases livestock growth, stocking rate (Shelton and Dalzell, 2007), soil carbon (Radrizzani *et al.* 2010), and reduces greenhouse gas emissions compared to tropical grass pastures (Taylor *et al.* 2016); thus enhancing farm-productivity and profitability (Bowen *et al.* 2016).

The configuration of the leucaena hedgerow silvopastoral system adopted in Queensland is based on practical grazer experience. However, there are no empirical data to help manipulate competitive interactions to optimise the yield of either the leucaena or grass components of the system. In Australia, density of commercial plantings of leucaena varies from 1,000 to 13,000 trees ha⁻¹ depending on density within the hedgerow, hedgerow spacing and whether single or double hedgerows are used (Radrizzani *et al.* 2010). In other regions of the world, the plant density of leucaena is often much higher at over 10,000 trees ha⁻¹ in pastures grazed for beef and dairy production (Murgueitio *et al.* 2011; Murgueitio *et al.* 2016). A better understanding of the above- and below-ground interactions between leucaena and grasses is required to optimise the design and management of leucaena-grass pastures.

In agroforestry systems, there is an imbalance of knowledge between above-ground versus below-ground interactions between tree and crop components. Most below-ground studies have focused on soil fertility changes, allelopathy and water uptake (Jonsson *et al.* 1988; Rao *et al.* 1993; van Noordwijk *et al.* 2004). These studies have revealed that roots of both trees and crops were concentrated in the topsoil and fine root biomass decreased with soil depth, although tap and lateral roots exploited greater depths of the soil profile (Jonsson *et al.* 1988; Dhyani *et al.* 1990). Maximum reported root depth in leucaena-grass pastures have varied from 2.6 m in 38-year-old leucaena growing in soil with a physical root barrier, to 6 m under 5-10 year-old leucaena-pastures grown in a deeper soil (Poole, 2001). However, approximately 60% of total root biomass was reported to be in the top 1 m of the soil profile (Poole, 2001; Akinnifesi *et al.* 2004; Radrizzani, 2010).

A general hypothesis in agroforestry systems is that trees can access water resources deeper in the soil profile than crops or pastures would otherwise exploit, leading to a degree of complementarity in their use of resources (Cannel *et al.* 1996; Schroth, 1999). However, it is also recognized that there is an overlapping shallow root zone where there is competition for water and nutrients.

The aim of this study was to determine the effect of leucaena plant density and grass competition on root distribution, soil water use patterns and the resulting water use efficiency (WUE) of both species.

6.2 Materials and methods

6.2.1 Experimental design

The experimental site and design were described in Chapter 5. Briefly, leucaena cv. Tarramba was planted on 27 November 2013 at 10 different densities using a Nelder fan design (Nelder, 1962) at the University of Queensland, Gatton Campus, Gatton, Queensland, Australia (27.54°S, 152.34°E). Twelve concentric rings which contained 16 trees (spokes) were planted at radii (spoke length) ranging from 0.6 to 37.6 m giving densities from 100 to 80,000 trees ha⁻¹ (Table 6.1, Fig. 6.1). The outer and the innermost ring, as well as spokes 1, 5, 9, 13, were used as guards. On 11 March 2014, two quarters of the experiment were sown to *Chloris gayana* Kunth. (Rhodes grass cv. Finecut). A weather station was installed in the experimental area to monitor daily solar radiation, temperature, humidity, wind speed and rainfall. Daily potential evapotranspiration (ET₀) was calculated using the Penman-Monteith equation (Allen *et al.* 1996). The methods and results for tree and grass above-ground interactions (biomass, leaf area, leaf area index, plant nutrition and light interception) were reported in Chapter 5.

Table 6.1: Radial (spoke) length, ring tree spacing and associated tree density and growing space of the Nelder fan experiment.

Radius	Tree density (tree ha ⁻¹)	Growing space (m ²)	Spacing within rings (m)	Spacing within spokes (m)
r_0	-----	-----	-----	-----
r_1	80,000	0.13	0.36	0.28
r_2	38,065	0.26	0.52	0.41
r_3	18,112	0.55	0.75	0.60
r_4	8,618	1.16	1.09	0.87
r_5	4,100	2.44	1.58	1.25
r_6	1,951	5.13	2.29	1.82
r_7	928	10.77	3.31	2.64
r_8	442	22.64	4.80	3.82
r_9	210	47.58	6.96	5.54
r_{10}	100	100.00	10.09	8.04
r_{11}	-----	-----	-----	-----

**Plate 6.1:** Aerial view of the Nelder fan (31/10/14) 338 days after establishment of leucaena trees and 234 days after establishment of Rhodes grass.

6.2.2 Measurement of water use

Soil water content (SWC) was monitored from 5 November 2014 to 17 March 2016 using a neutron moisture meter (NMM) (503 DR, CPN International Inc., Martinez, CA, USA). Forty-eight aluminum access tubes 50 mm in diameter and 4 m long were installed in October 2013, before the leucaena was planted. For this experiment, four densities were selected for intensive study corresponding to 100, 928, 8,618 and 80,000 trees ha⁻¹. Monitoring occurred from November 2014 to May 2016 (571 days) once the leucaena and Rhodes grass were considered to be fully established.

The access tubes were installed at the 4 leucaena densities, with and without grass competition, with two replicates. At the high density (80,000 trees ha⁻¹), four tubes were located 0.2 m from trees corresponding to

the midpoint between trees. At densities of 8,618 trees ha⁻¹, eight tubes were located at 0.2 m and 0.5 m (midpoint) from trees. At 928 trees ha⁻¹, 12 tubes were installed at 0.2, 0.5 and 1.6 m (midpoint) from trees. At the lowest density (100 trees ha⁻¹), 20 tubes were installed at 0.2, 0.5, 1.6, 2.5 and 5 m (midpoint) from trees (Table 6.2). Four tubes were installed in bare soil immediately adjacent to the site.

A linear regression between the NMM readings and soil volumetric water content was determined (R²= 0.8). During October and early November 2014, solid set sprinkler irrigation was applied for two weeks. Data from 35 observation periods were recorded during the study. At each period, SWC was measured at depths of 0.15, 0.45, 0.85, 1.35, 1.85, 2.35, 2.85, 3.25 and 3.75 m in the soil profile.

Table 6.2: Summary of number and location (●) of NMM tubes installed at different densities of leucaena grown with and without grass competition.

Treatment	Density (trees ha ⁻¹)	Tubes (n)	Distance from the tree (m)				
			0.18	0.54	1.6	2.5	5
Without grass competition	80,000	2	●	-----	-----	-----	-----
With grass competition	80,000	2	●	-----	-----	-----	-----
Without grass competition	8,618	4	●	●	-----	-----	-----
With grass competition	8,618	4	●	●	-----	-----	-----
Without grass competition	928	6	●	●	●	-----	-----
With grass competition	928	6	●	●	●	-----	-----
Without grass competition	100	10	●	●	●	●	●
With grass competition	100	10	●	●	●	●	●
Bare soil	---	4					

6.2.3 Evapotranspiration and water extraction

Actual evapotranspiration (ET_a) in mm d⁻¹ was calculated using a water balance equation (Equation 1) for each period of observation from November 2014 to May 2016.

$$ET_a = P + I - D - R - \Delta W \text{ (Equation 1)}$$

Where P and I correspond to entry of water as rainfall and irrigation, respectively, both expressed in mm d⁻¹, D is deep drainage (mm d⁻¹), R is runoff (mm d⁻¹) and ΔW is soil water change for the period of time. For this study, deep drainage and runoff were considered to be negligible.

For a better estimation of ET_a, the variables in equation 1 were subdivided into various components (Equation 2):

$$ET_{a,i} + ET_{a,nd} + ET_{a,d} = P_i + P_{nd} + P_d - D - R - \Delta W_{nd} - \Delta W_d \text{ (Equation 2)}$$

Where the subscript *i* indicates intercepted rainfall water, *nd* indicates water not detected by NMM, *d* indicates water that is detected by NMM, P_i is the amount of rainfall intercepted by foliage, P_{nd} is the amount of rain water that is held in the surface soil and not detected by NMM and P_d is the amount of bulk rain water that enters the soil and is detected by NMM. The assumptions were that that P_i = 3 mm, P_{nd} = 5 mm and P_d = P - 8 mm based on the H2OB water balance model description (Murtagh, 2012).

And if:

- P ≤ 3 then P_i = P, P_{nd} = 0, P_d = 0
- If 3 < P < 8, then P_i = 3, P_{nd} = P-3, P_d = 0
- If P > 8, then P_i = 3, P_{nd} = 5, P_d = P-8

Evapotranspiration and water extraction (WE) were calculated for each tube and for each tree density. These methodologies have been described fully by Eastham and Rose (1988). Briefly, water extraction by roots varied vertically (by depth), laterally (by radial distance from the tree) and with time. The volume of water in the soil can be assumed to be contained in a cylinder centred on the tree and with a radius which varies with

the density under study. Therefore, the cylindrical volume can be considered as a continuous nest of hollow cylinders at a fixed depth (4 m) and radius r and $r + \delta_r$ ($\delta_r = 0.01$ m). Values of actual evapotranspiration were calculated for each tube (ET_{ar}) and by linear interpolation to maximum radius (r_m , midpoint). Finally, ET_a for a tree density was calculated by summing ET_{ar} for each ring (Equation 3).

$$ET_a = \int_0^{r_m} ET_{ar} \pi[r^2 - (r - \delta_r)^2] / \pi r_m^2 \quad (\text{Equation 3})$$

Patterns of water extraction were studied through 3 drying cycles which corresponded to the periods 5 November 2014 – 13 November 2014 (8 days); 4 March 2015 – 13 March 2015 (9 days) and 13 January 2016 – 21 January 2016 (8 days). These periods were selected with the following criteria: rainfall did not occur, canopy size of leucaena and Rhodes grass was well-developed and there was high evapotranspiration demand. For each period, rate of water extraction was calculated using the same principle for the calculation of ET_a previously explained. Therefore, rate of water extraction for each depth was calculated by summing the water uptake for each ring to a determined depth under study (Equation 4).

$$WE = \int_0^{r_m} WE_r \pi[r^2 - (r - \delta_r)^2] / \pi r_m^2 \quad (\text{Equation 4})$$

6.2.4 Root measurements

Soil coring was used to directly measure root abundance of leucaena and Rhodes grass to 4 m depth. Forty-eight soil cores were collected from 14 to 16 June 2016; samples were taken 0.1 m adjacent to the NMM aluminum tubes (Table 6.2). Soil core samples were taken using a hydraulic soil corer mounted on a tractor using sampling tubes to 4 m depth. Tubes were steel alloy with an internal diameter of 50 mm and a cutting tip at the base of 42.5 mm, which allowed intact soil entry of the tube with minimal soil disturbance. Then the intact soil core was removed and placed in 4 half PVC tubes to expose soil cores without disturbance. Each core was broken at 0.1 m increments, the broken face observed, and the numbers of live roots of leucaena and Rhodes grass were counted. The roots of leucaena were brown-yellow in colour while Rhodes grass roots were white in colour. The presence of roots in each horizon was recorded in the following size categories: very fine <1 mm, fine 1–2 mm, medium 2–5 mm and coarse >5mm. As suggested by Wasson *et al.* (2014), the cores were broken with a snapping action instead of being cut with a knife as the aim was get best root exposure. For the purpose of analysis, root abundance was expressed as number of roots per 0.01 m² every 0.1 m depth.

6.2.5 Water use efficiency

Water use efficiency (WUE) was calculated as the ratio of accumulated above-ground biomass of leucaena and/or Rhodes grass (kg DM year⁻¹ ha⁻¹) to the total water consumed expressed as accumulated evapotranspiration (mm) (Sinclair *et al.* 1984; Hatfield *et al.* 2001). Data for above-ground biomass of leucaena without grass and biomass of leucaena grown with grass (kg DM year⁻¹ ha⁻¹) were calculated as described in Chapter 5. WUE was calculated at 4 leucaena densities with and without grass competition for two periods in 2014–2015 (from 10 October 2014 to 21 January 2015) (103 days) and 2015–2016 (12 October 2015 to 02 February 2016) (113 days).

6.2.6 Statistical analysis

Although the common statistical method used for Nelder fan experiments has been least square (OLS) regression to model relationships between density and observable variables (Cameron *et al.* 1989; Parrot *et al.* 2012), mixed-effect models can also be used on Nelder fan designs due to the hierarchical and spatial correlation of the data (Fox *et al.* 2001; Parrot *et al.* 2012). Mixed models contain fixed-effects that account for the relationship between dependent and independent variables; random-effects account for variation associated with sampling area (West *et al.* 2007; Parrot *et al.* 2012). For this study, we used mixed model analysis, where grass and no grass competition (cover) were used as random-effects, while fixed-effects were leucaena density (for analysing relative evapotranspiration water uptake and root abundance), soil depth (for analysing water uptake and root abundance), lateral distance (for analysing root abundance) and time (for analysing relative evapotranspiration). Statistical software used was GenStat 17 Edition (VSN International Ltd, Hemel Hempstead, UK).

6.3 Results

Rainfall events were concentrated from November 2014 to May 2015 (503 mm) and November 2015 to May 2016 (457 mm) (Fig. 6.1). The average maximum and minimum temperatures were 31.9 and 19.2°C in the hottest month (January) and 21.2 and 5.4°C in the coolest month (July). Average annual potential evapotranspiration was 5.4 mm d⁻¹.

6.3.1 Soil water content and evapotranspiration

Over the 571 days of study, the average soil water content varied from 1,031 to 700 mm according to density of leucaena and grass competition. Lower leucaena densities started and finished with higher soil water content compared to treatments with medium to high leucaena density or treatments with grass competition (Fig. 6.1).

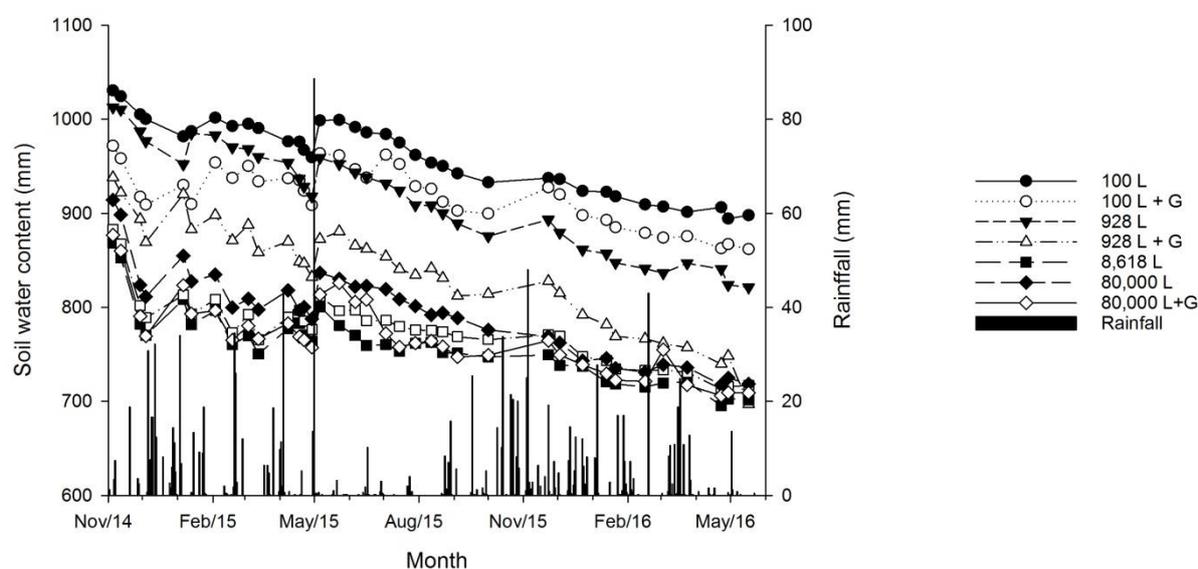


Fig. 6.1: Average soil water content during the period of study (mm) measured at four leucaena tree densities (100, 928, 8,618 and 80,000 trees ha⁻¹) grown with (L+G) and without grass competition (L). Rainfall events are presented as bars.

Relative evapotranspiration (E_t/E_{t_0}) from November 2014 to May 2016 under each of four leucaena densities grown with and without Rhodes grass competition is shown in Fig. 6.2. The highest values were recorded during December 2014 (0.71 to 1.25) and March 2016 (0.55 to 0.96). The lowest values of E_t/E_{t_0} were registered for leucaena without grass competition and ranged from 0.36 to 0.41 for densities of 100, 928 and 8,618 trees ha⁻¹. The maximum average relative evapotranspiration for the experimental period (1.25) was registered for leucaena at 100 trees ha⁻¹ grown with Rhodes grass and for leucaena plants grown at 80,000 trees ha⁻¹ (Fig. 6.2).

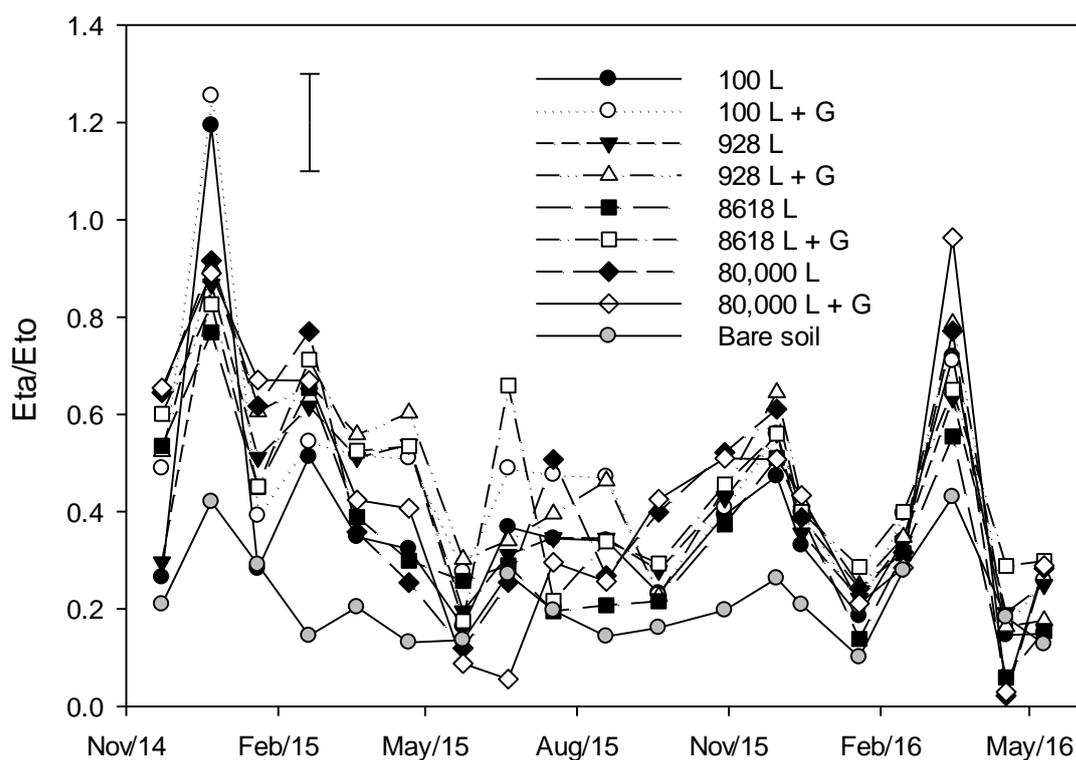


Fig. 6.2: Average relative evapotranspiration (ET_a/ET_o) per month at four densities of leucaena (100, 928, 8,618 and 80,000 trees ha^{-1}) grown with (L+G) and without grass competition (L). ET_a/ET_o for bare soil is also presented. The error bar indicates LSD ($P=0.05$) based on the analysis of all 8 treatments.

The cumulative evapotranspiration was calculated for each leucaena density. Highest values were recorded for leucaena grown with Rhodes grass or at high density (80,000 trees ha^{-1}). When leucaena plants were grown without grass competition, the cumulative evapotranspiration compared to the same densities with grass competition were reduced by 19%, 11%, 16% and 0.08% at 100, 928, 8,618 and 80,000 trees ha^{-1} respectively (Fig. 6.3). Cumulative evapotranspiration for bare soil was 557 mm.

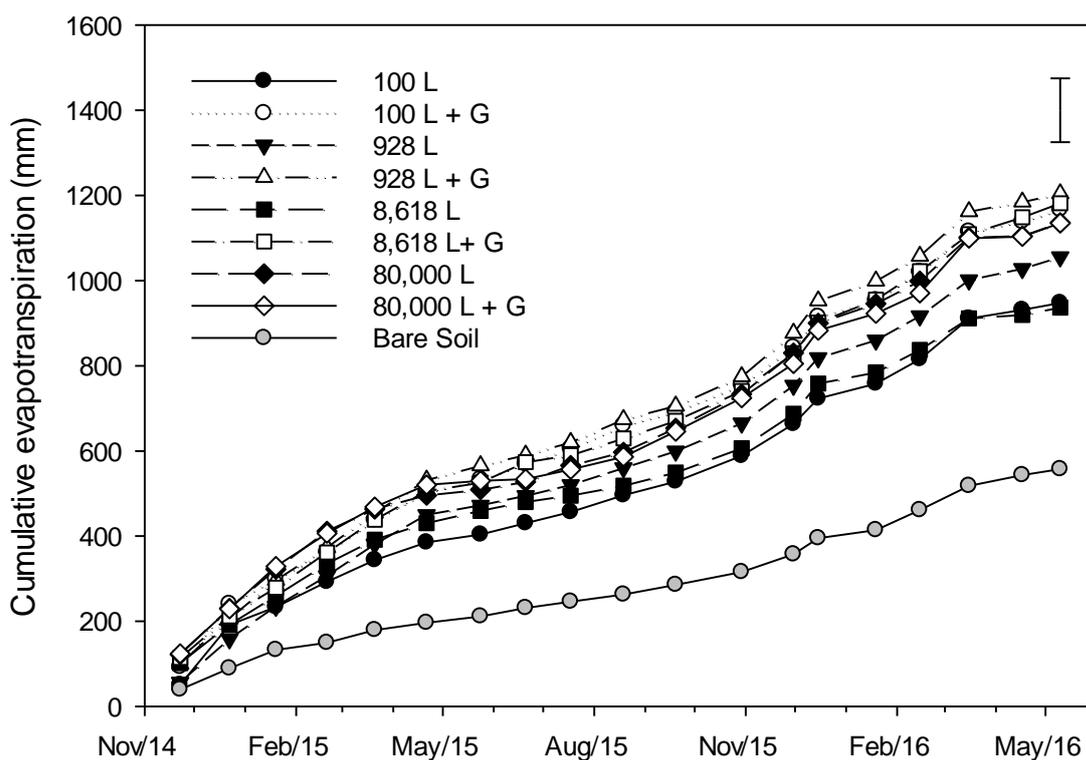


Fig. 6.3: Average cumulative evapotranspiration per month (mm) at four leucaena tree densities (100, 928, 8,618 and 80,000 trees ha⁻¹) grown with grass (L+G) and without grass competition (L) and for bare soil. The error bar indicates LSD ($P=0.05$) based on the analysis of all 8 treatments.

6.3.2 Water extraction

Overall, the average rate of water extraction (mm d⁻¹) was greatest in the surface soil zone and reduced with depth (Fig. 6.4). There was an interaction between grass competition, leucaena density and depth of water extraction ($p<0.05$). Differences between treatments due to grass competition were more evident at low leucaena density and became less significant as leucaena density increased (Fig. 6.4). At 100 trees ha⁻¹, leucaena grown with Rhodes grass registered high rates of water extraction from the 0–1.4 m deep soil layer (Fig. 6.4a), while at the same leucaena density without grass competition, ~70% less water was extracted from the soil profile. At 928 trees ha⁻¹, water extraction rates were higher in the upper soil profile (0–0.6 m depth) when leucaena was grown with Rhodes grass competition. These differences reduced with increasing soil depth. When leucaena was grown at high densities, differences in water uptake, with and without grass competition, were negligible. Water extraction rates followed the same pattern being higher in the upper soil profile and decreasing with soil depth.

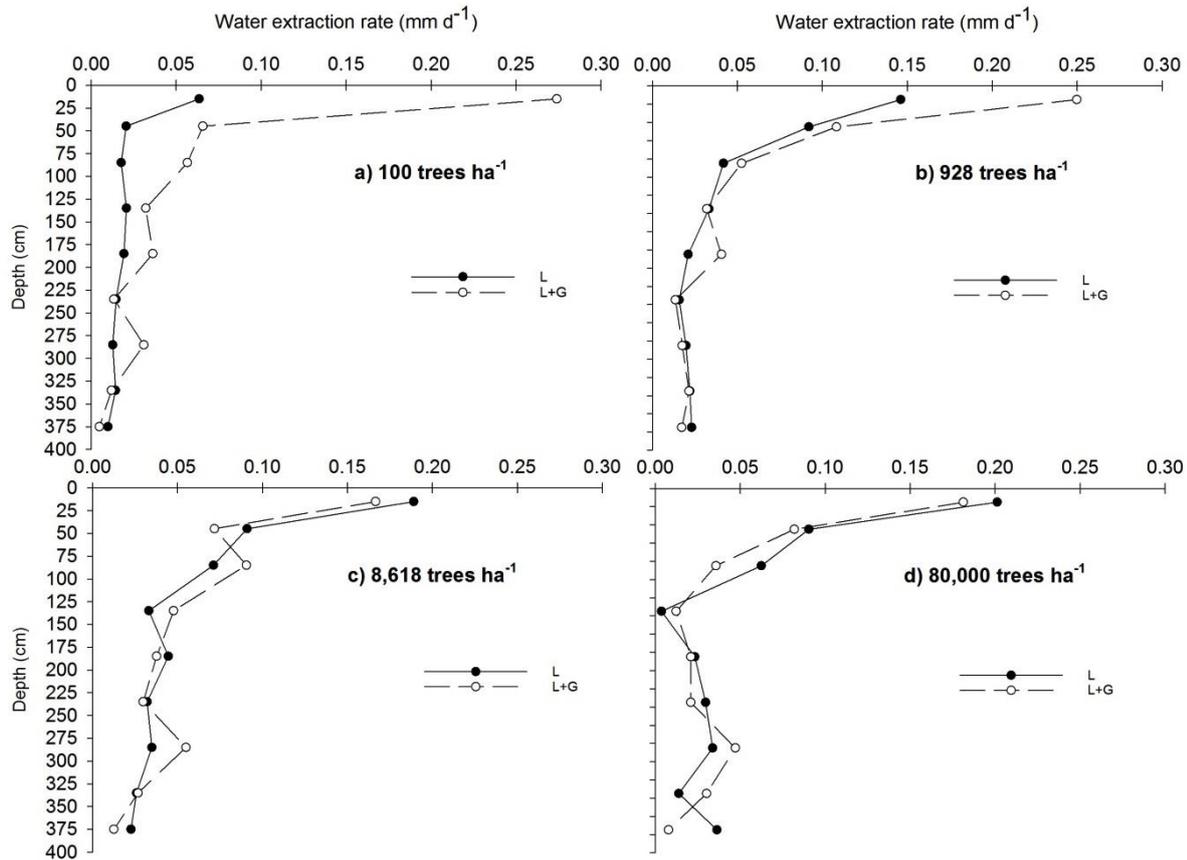


Fig. 6.4: Average water extraction rate (mm d^{-1}) by soil depth measured from 5–11 November 2014, 4–13 March 2015 and 13–21 January 2016 at four densities of leucaena grown with grass (L+G) and without grass (L) competition.

6.3.3 Root distribution and abundance

More than 99% of the roots counted were classified as fine roots (<1 mm diameter). Overall, root abundance was higher in the top soil and decreased with depth to 4 m ($P < 0.01$) regardless of tree density and grass competition.

At 100 trees ha^{-1} , there was a significant interaction between grass competition and rooting depth ($P < 0.001$). Leucaena grown without grass competition had 43% more roots in the upper soil profile (0–0.5 m depth) than when grown with Rhodes grass (Fig. 6.5a and 6.5b). Overall, leucaena root abundance decreased with increasing soil depth and lateral distance ($P < 0.001$). Lateral spread of leucaena roots reached 5 m when grown without grass competition and only 2 m when grown with Rhodes grass competition (Fig. 6.5a and 6.5b).

At 928 trees ha^{-1} leucaena root abundance did not vary with and without grass competition or depth ($P = 0.08$), although a trend of greater root abundance was observed at 0–0.5 m depth when leucaena was grown without grass competition (Fig. 6.5c). Greater ($P < 0.01$) root abundance was observed at depth (~2.6–3 m) for leucaena plants grown with Rhodes grass (Fig. 6.5d).

At 8,618 trees ha^{-1} , root abundance decreased with increasing soil depth ($P < 0.01$). Leucaena grown without grass competition had ~40% greater root abundance at 0.5 m compared to leucaena grown with Rhodes grass, however these differences were weakly significant ($P = 0.08$) (Fig. 6.5e and 6.5f). Higher root abundance of leucaena was observed at 4 m depth for leucaena plants grown without grass competition (Fig. 6.5e).

At 80,000 trees ha^{-1} , leucaena root abundance at 0.2 m, with and without grass competition, was similar as grass growth was negligible (Fig. 6.5g and 6.5h).

Root abundance observed at 0.2 m from leucaena trees increased with density of leucaena when grown with grass competition ($P < 0.001$). At 80,000 trees ha^{-1} , root abundance was 37 and 45% greater than observed at 8,618 and 928 trees ha^{-1} respectively (Fig. 6.5).

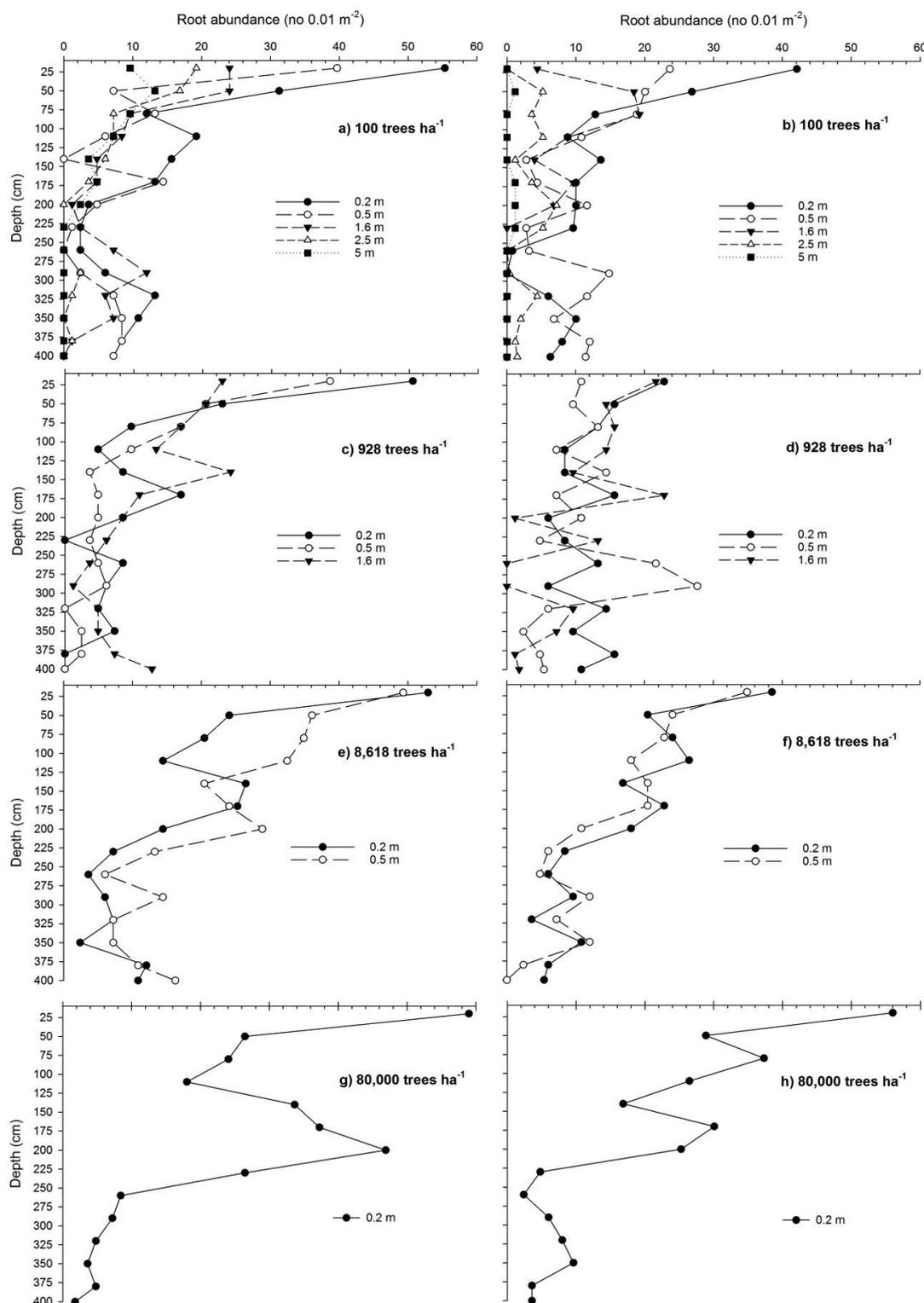


Fig. 6.5: Root distribution patterns of leucaena grown at 4 densities with (L+G) and without (L) grass competition measured at different depths (0.2 to 4 m) and distances from leucaena trees (0.2 to 5 m): (a) 100 trees ha^{-1} L, (b) 100 trees ha^{-1} L+G, (c) 928 trees ha^{-1} L, (d) 928 trees ha^{-1} L+G, (e) 8,618 trees ha^{-1} L, (f) 8,618 trees ha^{-1} L+G, (g) 80,000 trees ha^{-1} L and (h) 80,000 trees ha^{-1} L+G.

Rhodes grass had higher root abundance than leucaena. For example, at 0.2 m depth Rhodes grass root abundance was 60-400 per 0.01 m⁻² compared to 25-56 per 0.01 m⁻² for leucaena. Rooting patterns of Rhodes grass varied with leucaena density ($P<0.001$), depth ($P<0.001$) and distance from the tree ($P<0.023$). Overall, Rhodes grass root abundance decreased and became shallower with increasing leucaena density (Fig. 6.6).

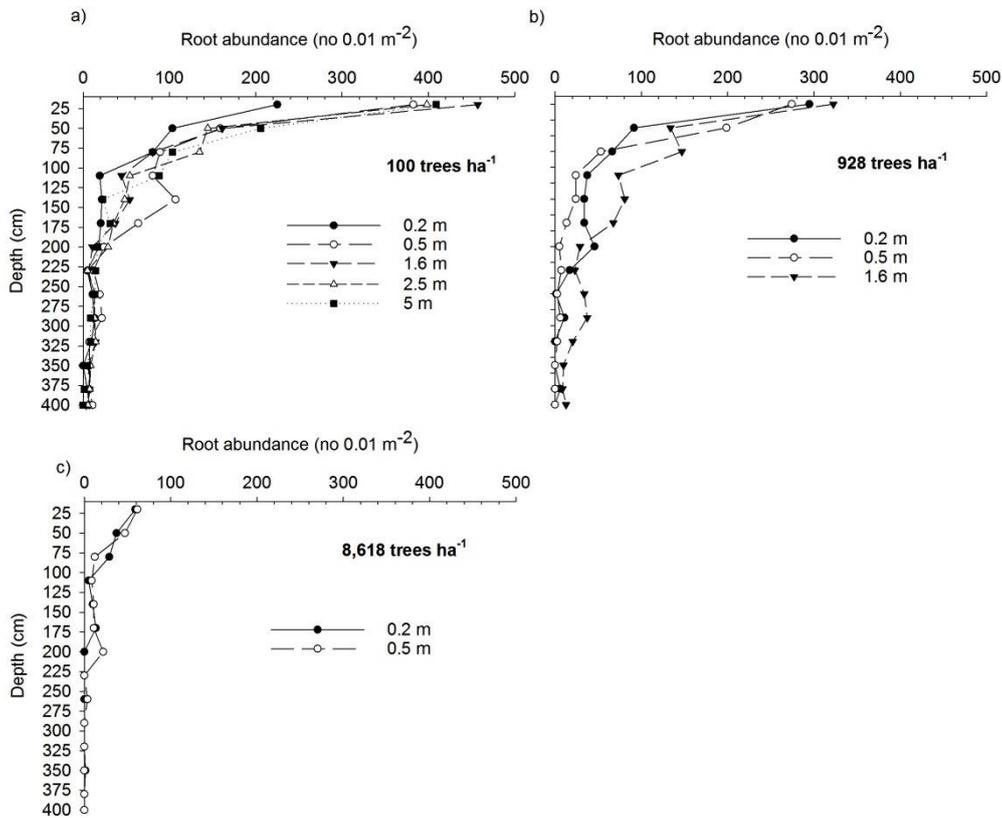


Fig. 6.6: Root distribution patterns of Rhodes grass grown with different leucaena tree density measured at different depths (0.2 to 4 m) and distances from leucaena trees (0.2 to 5 m): a) 100 trees ha⁻¹; b) 928 trees ha⁻¹ and c) 8,618 trees ha⁻¹.

6.3.4 Water use efficiency

Overall, WUE of the leucaena component increased with density and age (Table 6.3). Differences in WUE between leucaena grown with and without grass competition were evident at the lowest leucaena density in both periods. The presence of Rhodes grass increased water use more efficiency. Maximum WUE was recorded in period 2015-2016 at the highest leucaena densities regardless of grass competition which was ~55% higher than in 2014-2015.

Table 6.3: Water use efficiency estimated for 4 leucaena plant densities grown with and without Rhodes grass for periods in 2014-2015 (10/10/14 to 21/01/15) (103 days) and 2015-2016 (12/10/15 to 2/02/2016) (113 days).

Tree density (trees ha ⁻¹)	Accumulated ET _a (mm)	Above-ground biomass of leucaena (kg DM ha ⁻¹)	Above-ground biomass of Rhodes grass (kg DM ha ⁻¹)	WUE (kg DM mm ⁻¹)
2014-2015				
a) Leucaena without grass competition				
100	240	448	-----	1.9
928	222	2843	-----	12.8
8,618	284	6989	-----	24.6
80,000	345	9615	-----	27.9
b) Leucaena with grass competition				
100	324	176	6956	22.0*
928	303	1,958	5574	24.9*
8,618	300	6,593	1386	26.6*
80,000	348	10,262	0	29.5*
2015-2016				
a) Leucaena without grass competition				
100	260	1819	-----	7.0
928	288	6840	-----	23.8
8,618	262	13507	-----	51.5
80,000	311	20591	-----	66.3
b) Leucaena with grass competition				
100	299	790	4931	19.1*
928	314	4,961	4909	31.5*
8,618	283	11,921	212	42.8*
80,000	291	19,120	44	65.9*

*Indicates WUE was calculated as sum of leucaena and Rhodes grass biomass divided by cumulative ET_a

6.4 Discussion

6.4.1 Effect of leucaena density on root distribution and water extraction of both species

As it was hypothesized, leucaena plant density influenced rooting patterns and water extraction of leucaena and Rhodes grass. Greater and deeper leucaena root abundance was recorded at 80,000 trees ha⁻¹. At lower densities, greater lateral spread of leucaena roots was recorded. Budisantoso (2005) measured the effect on rooting depth of three leucaena planting densities. He found that root index (a measurement of abundance) was higher near the soil surface, and decreased with depth. Eastham and Rose (1990), in their research on the effect of tree density on root distribution, also found that with increasing tree density, the root systems were more dense and deeper.

6.4.2 Effect of grass competition on root distribution of leucaena

In the Nelder fan experiment, although root biomass of leucaena and Rhodes grass was not measured, root abundance data demonstrated how leucaena and Rhodes grass root systems varied according to leucaena

density, and how grass competition reduced the lateral spread of leucaena at lower density from 5 to 2.5 m. Several authors reported lateral spread of leucaena roots when grown with different crops varied from 1.4 to 2.5 m (Toky and Bisht, 1992; Dyani *et al.* 1999; Govindarajan 1996). According to Schroth (2003), trees can modify root architecture such as lateral spread as a mechanism to reduce excessive intra- and interspecific competition forming a stratified vertical root development near to the tree. In addition, there is evidence that grasses with competitive root systems reduced lateral spread and increased depth of root systems of trees when grown together (Neves *et al.* 1998; Shaller *et al.* 2001; Schroth, 2003). In a study of the interaction between *Leucaena leucocephala* and *Manihot esculenta* (cassava), Gosh *et al.* (1989) reported that maximum lateral spread of leucaena at 36 months was 3.46 m and it was significantly reduced to 1.9 m when grown with cassava.

6.4.3 Effect of leucaena competition on root distribution of Rhodes grass

Rhodes grass root abundance was drastically reduced by increased leucaena density. Similar results were found by Eastham and Rose (1990), who reported that with increasing tree density pasture root biomass, was drastically reduced. At medium and low tree density, pasture root biomass was found to be maximum at the midpoint (6.2 m) between rows. It is suggested that this change was associated with a combination of above- and below-ground interspecific competition due to increasing shade by the leucaena canopy and increasing root abundance of leucaena. Although, Rhodes grass has been reported to tolerate shade (Dunn *et al.* 1994; Wilson 1996), high shade levels (over 50%) were measured in the current study and a drastic decline in above-ground biomass was measured at densities of 8,618 trees ha⁻¹ in the same experiment (Chapter 5). The decline in above-ground biomass, plus the suggestion that plants growing under shade change their biomass allocation to above-ground structures (Brown, 1963; Evans and Porter, 2001; Fernandez *et al.* 2004; Pachas, 2010) could be the explanation for the major changes in root abundance measured in this experiment.

6.4.4 Complementarity and competition for water resources

Changes in rooting patterns have a huge impact on below-ground competition for water resources, therefore on inter and intraspecific competition. It is assumed that there is spatial complementarity of use of the resources when trees and grass are combined, as tree roots can acquire water deeper in the soil strata (Cannel *et al.* 1996). In a study of vertical root distribution of multipurpose tree species, Jonsson *et al.* (1988) clarified the definition of “trees with deeper roots”, because it is not clear if it infers that trees have most of their root biomass deep in the soil, or whether some roots reach deeper levels while the bulk of roots remain near to the surface. Clearly, in the Nelder fan experiment, leucaena had deeper root abundance than Rhodes grass suggesting a complementary use of water resources; however, the great abundance of fine roots of leucaena and Rhodes grass in the upper soil levels suggested a high level of competition for water resources. Rhodes grass competed strongly with leucaena for water resources due to its greater root abundance, especially at low densities of leucaena. In addition, it is suggested that the high level of competition for water resources was the main reason for the reduction by 50-70% of the above-ground biomass of leucaena when grown with Rhodes grass (Chapter 5). In a recent study of water use and root patterns of leucaena-Rhodes grass pasture, Pachas *et al.* (2016) reported greater water extraction in the upper soil profile with extraction decreasing with depth. In twin-row configurations (8 m spacing between paired hedgerows), leucaena trees extracted 75% of water from the upper 1.5 m of soil profile while water extraction by Rhodes grass (measured at midpoint -4 m- of leucaena alley) was 90% at the same depth. Pachas *et al.* (2016) (Chapter 3) showed the average depth of maximum water extraction was 2.2 m for leucaena and 1.8 for Rhodes grass during a wet year. However, water extraction at 4m depth was observed for both species in some months but mainly from leucaena plants.

The range of average total water extraction (641-799 mm year⁻¹) registered in our study was similar to that reported for a commercial leucaena-Rhodes grass pasture in Central Queensland, Australia during a wet year (Pachas *et al.* 2016). However, total water extraction was lower compared to that found by Narain *et al.* (1998) (average rainfall of 1037 mm year⁻¹) who measured 1528 and 1397 mm year⁻¹ in sole leucaena and leucaena-grass pasture respectively.

6.4.5 Effect of leucaena density on WUE

The findings of this study confirmed the hypothesis that at low densities, leucaena-grass pasture will have higher WUE than sole leucaena, but this difference would be reduced with increments of leucaena density. Budisantoso (2005) found that WUE increased with plant density and that leucaena had the greatest WUE (average 101 kg DM mm⁻¹) compared to *Sesbania grandiflora* and *Gliricidia sepium*. Water use efficiency values recorded in the Nelder fan experiment were higher than 3.28 kg DM ha mm⁻¹ reported by Radrizzani (2010) for 8 year-old leucaena in Central Queensland. Values of WUE for leucaena-Rhodes grass pasture measured in the Nelder fan experiment at low leucaena density (13.8 kg DM mm⁻¹) were similar to 15.2 kg DM mm⁻¹ reported by Callow (2011) for pure Rhodes grass pasture in Southern Queensland and to 11.8 kg DM mm⁻¹ measured by Budisantoso (2005) in leucaena-buffel grass pasture in the Fitzroy Basin in Central Queensland. Increasing WUE with tree density was also reported by Eastham and Rose (1990), working with *Eucalyptus grandis* and *Setaria sphaceolata*, where WUE increased from 13 kg DM mm⁻¹ at low tree density to 46 kg DM mm⁻¹ to highest tree density.

6.5 Conclusion

The result of the Nelder fan experiment demonstrated that leucaena tree density and grass competition strongly influenced rooting patterns, water extraction and water use efficiency of leucaena and Rhodes grass. Most roots of leucaena and Rhodes grass and therefore water extraction were observed in the upper soil profile (<1.5m depth) indicating high competition for resources. There was some complementarity as leucaena was able to extract water from deeper in the soil profile, once surface soil moisture was depleted.

In terms of WUE, leucaena grown at high densities had the highest WUE. Nevertheless, when leucaena was grown at lower densities (<8,618 trees ha⁻¹), the combination with Rhodes grass increased WUE compared with leucaena grown without grass.

7 : Effects of within-row plant densities on water use and root patterns of *Leucaena leucocephala*

7.1 Introduction

The multipurpose tree legume leucaena [*Leucaena leucocephala* (Lam.) de Wit ssp. *glabrata* (Rose) Zarate] is widely used for ruminant feeding in subtropical and tropical regions. In Queensland Australia, leucaena hedgerows are combined with grasses (e.g. *Chloris gayana* Kunth, *Pennisetum ciliare* L.) resulting in highly productive grazing systems for beef production (Shelton and Dalzell, 2007).

In Queensland, planting density of broad-acre leucaena ranges between 1,000 to 8,000 trees ha⁻¹, depending on hedgerow spacing, which can be 4 to 15 m apart (Radrizzani *et al.* 2010); and the density of plants within the hedgerow which can be 0.1 to 1 m apart. Another factor is whether single or twin hedgerows 0.5–1 m apart are used (Shelton and Dalzell, 2007).

In leucaena-grass pastures, there have been relatively few studies of the above-ground interactions and almost no studies of below-ground attributes such as water use and root distribution (van Noordwijk *et al.* 2004). Budisantoso (2005) studied the effect of planting density on water use and water use efficiency (WUE) for three tree legumes *Leucaena leucocephala* cv. Tarramba, *Gliricidia sepium* and *Sesbania grandiflora* and showed that most water extraction occurred in the upper 1.2 m soil profile; however, it varied according to species and planting density and leucaena had the highest water use and WUE. He also found that plant density and species affected above-ground biomass; leucaena had the highest biomass followed by sesbania and gliricidia.

For the studies presented here, in another experiment, conducted as part of this overall research program, the effect of plant density on above- and below-ground interactions was investigated and is reported separately in Chapter 6.

The aims of the present study were to determine the effects of within-row leucaena plant density on above-ground biomass yield, water use and rooting patterns. The hypotheses were that higher within-row density will: (a) increase above-ground biomass of leucaena; (b) increase vertical and lateral rooting distance; (c) increase soil water extraction; and (d) over long dry-periods lead to greater access of deep soil water than leucaena planted at lower density.

7.2 Materials and methods

7.2.1 Location

The experiment was conducted at the University of Queensland research farm at Gatton (27.54°S, 152.34°E) in south-east Queensland, Australia. The region experiences a subtropical climate with an annual average rainfall of 772 mm, predominantly during the summer months with average maximum and minimum monthly temperatures for the hottest and coldest months of 31.6/19.3°C in January and 20.7/6.2°C in July. The experimental site was located on a Fluventic haplustoll (Isbell, 1996) that contains 5% coarse sand, 20% fine sand, 22% silt and 53% clay. A detailed soil description was reported from previous experiments at the same site (Powell, 1982; Mitchell *et al.* 2013; Zhou *et al.* 2014).

7.2.2 Experimental design

On 29 November 2013, *Leucaena leucocephala* cv. Tarramba seedlings were planted in single 6 m row plots under two automatic rainout shelters (Plate 7.1). A randomized block design with four replications and two densities within-row were tested: (a) low density (LD) 1 plant every 1 m and (b) high density (HD) 1 plant every 0.1 m. Rows were 8 m apart thus eliminating the possibility of between row competition. Soil water content (SWC) was monitored during 4 selected drying periods: period 1 (P1) from 19 March 2014 to 9 July 2014 (112 days), period 2 (P2) from 13 November 2014 to 11 February 2015 (94 days), period 3 (P3) from 4 March 2015 to 16 June 2015 (104 days) and period 4 (P4) from 7 November 2015 to 1 March 2016 (114 days).

For periods 1-3, irrigation was applied to field capacity at the commencement of the drying cycle; thereafter rainout shelters were used to prevent incoming rain until March 2016.



Plate 7.1: Aerial view of rainout shelter experiment (8 October 2014) 313 days after establishment of leucaena trees.

7.2.3 Above-ground biomass

Above-ground biomass per tree was determined by destructive harvests to ~1 m above-ground-level on 24 July 2014, 11 February 2015 and 3 March 2016. Detailed sampling methodology was described in Chapter 5. Leaf area was measured using a portable leaf area meter LI-COR 3200 (Li-Cor, Inc., Lincoln, NE).

7.2.4 Root abundance

Soil coring methodology was used to directly measure root abundance of leucaena to 4 m depth. Sixty four soil cores were collected from 2 to 5 March 2016 using a hydraulic soil corer mounted on a tractor. Samples were taken at four distances from the leucaena tree base: 0.2, 1, 2 and 3 m, on both sides and perpendicular to leucaena rows. Detailed methodology for root abundance measurement was described in Chapter 6. Briefly, steel alloy tubes with an internal diameter of 50 mm and a cutting tip at the base of 42.5 mm, which allowed minimal soil disturbance, were used to sample leucaena roots. Intact soil samples were removed to 4 half PVC trays to expose soil cores without disturbance. Each core was broken at 0.1 m increments, the broken face observed, and the number of live roots counted. Once the cores were broken, it was easy to distinguish and count leucaena roots (brown-yellow colour). The presence of roots in each horizon was recorded according to size categories: very fine <1 mm, fine 1–2 mm, medium 2–5 mm and coarse > 5mm. As suggested by Wasson *et al.* (2014), the cores were broken with a snapping action instead of being cut with a knife as the aim was get best root exposure. For the purpose of analysis, root abundance was expressed as number of roots per 0.01 m² every 0.1 m depth.

7.2.5 Soil water measurement

Volumetric soil water content was monitored using a neutron moisture meter (NMM, model CPN 503 DR Campbell Pacific Nuclear International Inc., USA) and portable capacitance probes (Diviner 2000 Sentek Pty. Ltd., Stepney South, Australia). A total of 24 aluminium access tubes, 50 mm in diameter and 4 m long, and 48 polyvinyl chloride (PVC) tubes, 56 mm in diameter and 2 m long, were installed before leucaena was planted to avoid disturbance during the experiment. In each plot, 3 aluminium access tubes were installed at 0.2, 0.5 and 1 m distance from the leucaena row, while 6 PVC tubes were installed at 0.2, 0.5, 1, 1.5, 2 and 3 m distance from the leucaena row and 1 m apart but parallel to NMM tubes. NMM readings (at 16 second

intervals) were obtained from 0.15, 0.45, 0.85, 1.35, 1.85, 2.35, 2.85, 3.25 and 3.75 m depths in the soil profile. For calibration purposes, NMM readings and soil volumetric content were measured when the access tubes were inserted into the soil profile and data were pooled with previous data from the same experimental site and a relationship between NMM counts and volumetric soil water content was developed ($R^2=0.84$, $n=98$). Diviner 2000 readings were obtained from a depth of 0.1 to 1.0 m at 0.1 m intervals. A calibration equation was developed between scaled frequency and volumetric soil water content ($R^2=0.92$, $n=48$). For the duration of the experiment, SWC measurements were taken every ~20 days.

Correlations between NMM and Diviner 2000 were carried out comparing volumetric soil water content measured on the same day. Paired data, measured in the same plot and distance from leucaena rows (0.2 and 1 m) and depth (0.15, 0.45, 0.85, 1.35 m), were plotted and a linear regression equation developed. As Diviner 2000 measurements were at 0.1 m depth intervals, SWC data used for correlation were obtained by averaging readings between 0.1–0.3, 0.4–0.50, 0.8–0.9 and 1.3–1.4 m depth.

7.2.6 Water extraction and plant available water

It is understood that a decrease in soil water content could be due to soil evaporation, plant water uptake, runoff or drainage. Nevertheless, for this experiment, runoff and drainage were considered negligible. Thus plant water extraction (mm) was calculated by subtracting the SWC between periods while water extraction rates (mm d^{-1}) were obtained by dividing by days between measurements (ΔT). For each period studied, rate of water extraction was calculated for each depth and lateral distance for both NMM and Diviner measurements.

Plant-available water capacity (PAWC) was calculated at each sampling depth from the difference between field capacity (FC) and permanent wilting point (PWP); these parameters were determined on a pressure plate apparatus at 10 kPa and 1,500 kPa respectively. Plant available water (PAW) was calculated as the difference in SWC between FC and PWP. Soil available water proportion (SAWP) was calculated as ratio between PAW and PAWC.

Overall, NMM data were used to determine the effect of density within-row on water use by depth, while Diviner 2000 data were used to determine the effect of water use according to lateral distance from leucaena trees.

7.2.7 Statistical analysis

Each period was analysed individually. Analysis of variance (ANOVA) of above-ground biomass and leaf area was performed to compare the effect of within-row spacing. Tukey's test was used to compare means between densities at the 95% confidence level. A linear mixed model analysis (REML procedure) (Piepho *et al.* 2004; Brian and Demetrio, 2009) was used to compare below-ground plant density (Density) effects on root abundance and water used by depth (Lay), distance (Dist) and time (Time). For analysis of root abundance and rate of water used, a random-effects model (Block.Plot)*(Lay.Dist) and a fixed-effects model were used (Block + Density)*(Lay.Dist). For analysis of the effect of time, soil water content and water used, the random-effects model used was (Block.Plot)*(Distance.Time), while the fixed-effects model used was (Block + Treat)*Dist*Time. Assuming a correlation between depth and distance of root abundance and soil water content, covariance structures for errors were considered in the model such as AR1 or AR2. Akaike's Information Criterion (AIC) was used to select the best fitting model. Linear regression analysis (GLM procedure) was used to determine the relationship between volumetric soil water data measured with NMM and Diviner 2000. All statistical analysis of ANOVA, REML and GLM were carried out using GenStat 17 Edition (VSN International Ltd, Hemel Hempstead, UK).

7.3 Results

7.3.1 Above-ground biomass and leaf area

Above-ground biomass and leaf area (LA), on an individual tree basis, varied according to within-row spacing; for all periods, individual trees at low density had greater biomass and leaf area ($P<0.001$). However, when above-ground biomass and LA were expressed per linear meter (kg DM m^{-1} and $\text{m}^2 \text{m}^{-1}$ respectively), these

variables were not significantly different except in the first period when plants were still young ($P>0.05$) (Table 7.1).

Table 7.1: Effect of within-row density on above-ground biomass and leaf area expressed per tree and per linear meter for periods 1 (112 days), 2 (94 days) and 3-4 (365 days).

Parameters	P1	P2	P3-P4
Individual tree above-ground biomass (kg DM tree ⁻¹)			
<i>Low density</i>	0.42 ^a	4.38 ^a	8.7 ^a
<i>High density</i>	0.19 ^b	0.48 ^b	0.8 ^b
Total above-ground biomass (kg DM m ⁻¹)			
<i>Low density</i>	0.42 ^b	4.38 ^{ns}	8.7 ^{ns}
<i>High density</i>	1.9 ^a	4.86 ^{ns}	8.2 ^{ns}
Individual tree leaf area (m ² tree ⁻¹)			
<i>Low density</i>	1.5 ^a	17.6 ^a	10.8 ^a
<i>High density</i>	0.9 ^b	1.95 ^b	1.2 ^b
Total tree leaf area (m ² m ⁻¹)			
<i>Low density</i>	1.5 ^b	17.6 ^{ns}	10.8 ^{ns}
<i>High density</i>	8.7 ^a	19.5 ^{ns}	12.3 ^{ns}

*Different letters within a column indicate that means are significantly different at 95% confidence level.

7.3.2 Rooting patterns

Root abundance of leucaena decreased with distance from leucaena trees ($P<0.001$) and with depth ($P<0.001$). As there were no significant effects of density within-row on water use ($P=0.305$) data were pooled (Fig. 7.1). Root abundance decreased with depth and lateral spread and roots reached beyond 4 m depth and 3 m laterally (Fig. 7.1a and 7.1b). Approximately, 75% of all fine roots were found in an arc that varied from 4 m depth and 1.8 m lateral spread (Fig. 7.1.b).

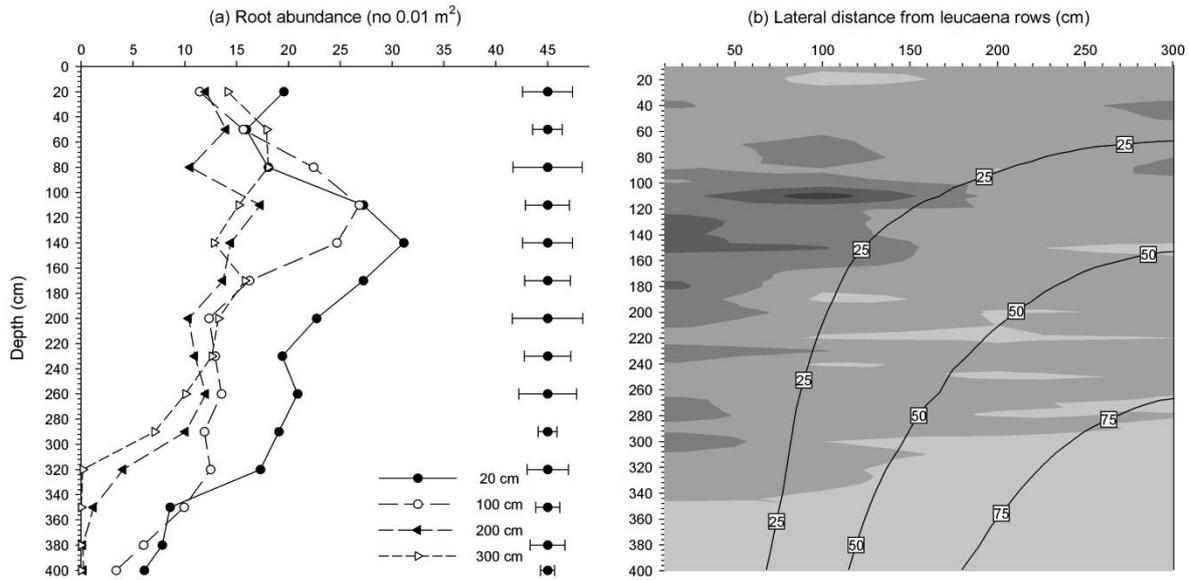


Fig. 7.1: (a) Average root abundance of leucaena measured to 4 m depth and at four distances from leucaena rows. Horizontal bars indicate standard errors; (b) Average vertical and lateral root abundance of leucaena; lines represent cumulative root abundance at 25, 50 and 75%. A dark colour indicates more root abundance.

7.3.3 Soil water content and plant available water (NMM data)

Comparison of data for volumetric soil water content measured by Diviner 2000 and NMM revealed a strong linear relationship ($R^2=0.92$) with $0.55 \text{ m}^3 \text{ m}^{-3}$ intercept and slope $0.96 \text{ m}^3 \text{ m}^{-3}$ (Fig. 7.2).

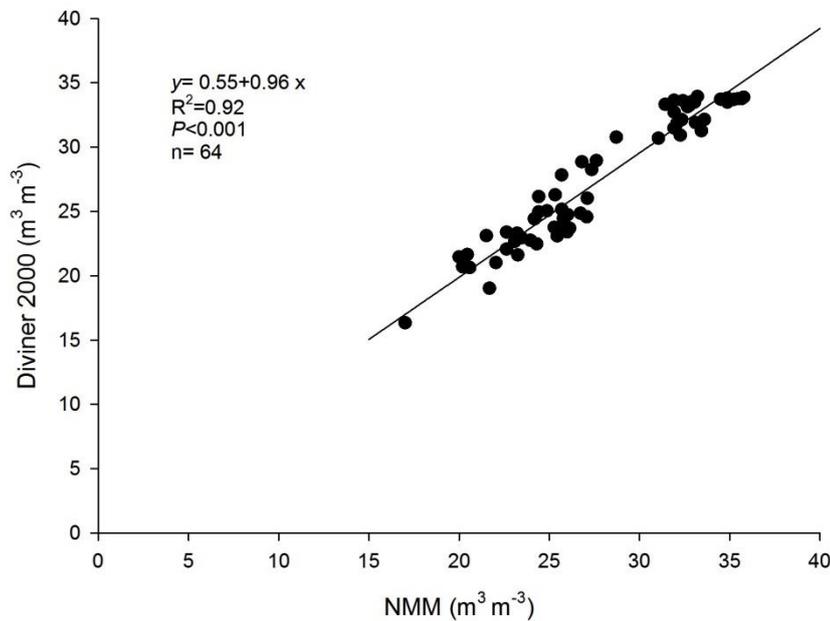


Fig. 7.2: Correlation between volumetric soil water content measured by Neutron Moisture Meter and Diviner 2000.

For all periods, total SWC to 4 m depth decreased with time from imposition of the dry spell ($P < 0.001$). Also, there were no significant effects ($P > 0.05$) of density and distance from the row (from 0.2 to 1 m) on SWC readings. Overall, highest soil water available and water proportion was observed at the beginning of P1, P2 and P3 and decreased with time to an average of ~ 0.45 SWP in the upper 2 m soil profile. Deeper in the

profile, the average SWP for P1, P2 and P3 was 0.64. For period P4, which was not irrigated at the commencement of sampling, average SWP was 0.45 and finished at ~ 0.33 SWP in the upper soil profile (0-2 m depth). Deeper in the soil profile (2 to 4 m depth), plant available water at the beginning of the period was 63% and decreased with time to ~ 0.42 SWP. An example of trends of soil water content and soil water proportion during the period of study, measured at 1 m distance from tree rows, is presented in Fig. 7.3.

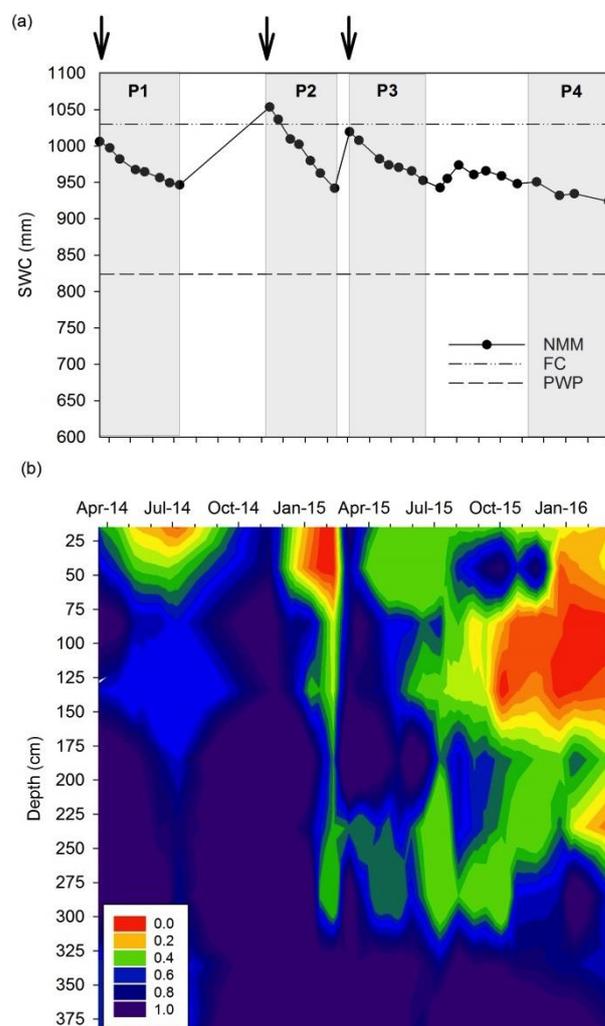


Fig. 7.3: (a) Trends of soil water content of leucaena growing at low tree densities within-row measured by NMM at 4 m depth; (b) Temporal patterns of vertical soil available water proportion (SWAP from 0 to 1) calculated for the same plot. Arrow indicates irrigation events at commencement of P1, P2 and P3. Field capacity (FC) and permanent wilting point (PWP) are indicated as lines

7.3.4 Vertical patterns of water extraction (NMM data)

Analysis of NMM data for all periods indicated that rate of water extraction varied with depth (0.15 to 4 m) ($P < 0.001$). As the effects of densities within-row and lateral distances from the tree (0.2, 0.5 and 1 m) were not significant ($P > 0.05$), data were pooled (Fig. 7.4). Water extraction was greater in the upper soil profile and decreased with depth. The second period was characterized as having greater water extraction compared to the other periods while P4 had the lowest water extraction rate (Fig. 7.4a, 7.4b, 7.4c and 7.4d).

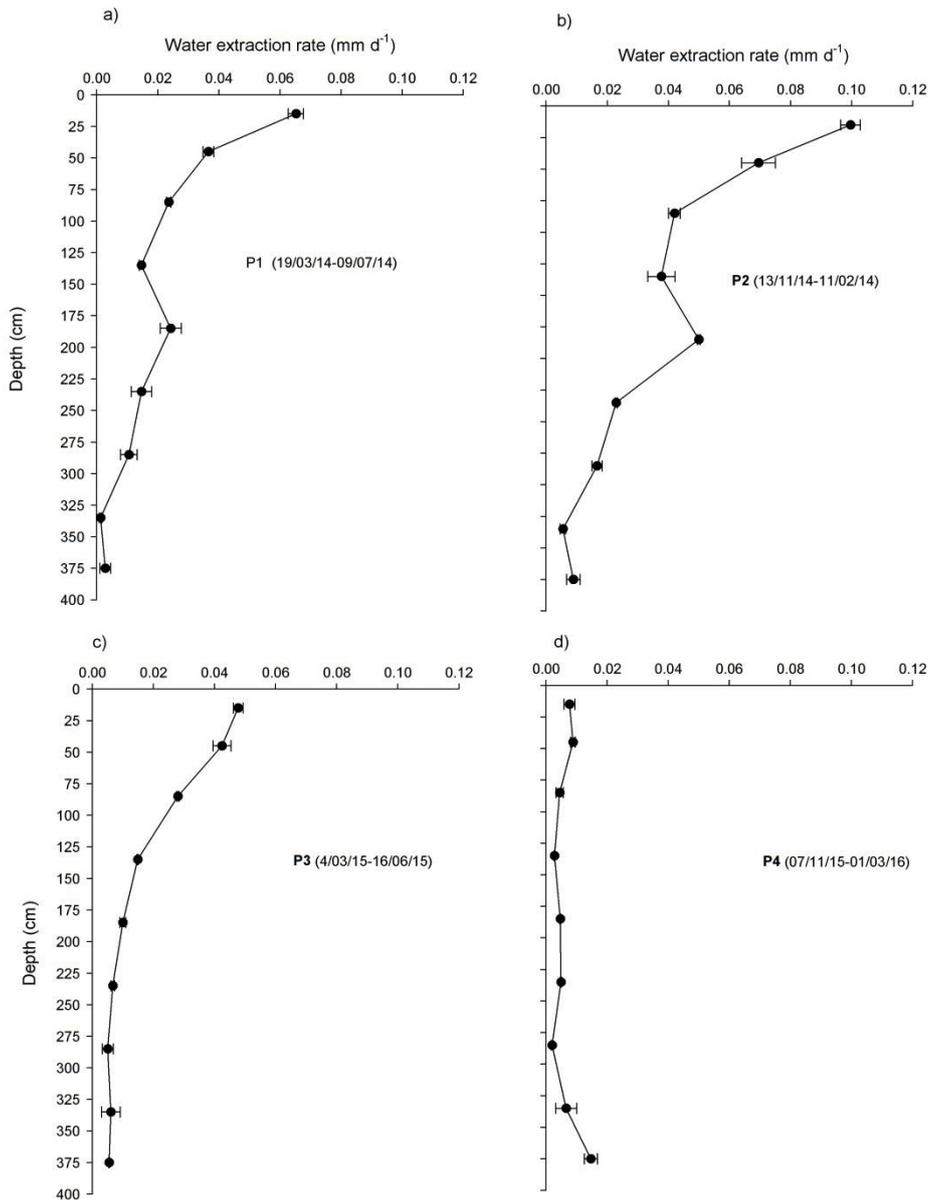


Fig. 7.4: Average water rate of extraction measured to 4 m depth and from 0.2 to 1 m lateral distance from leucaena trees measured during four dry-periods under rainout shelters: (a) period 1, (b) period 2, (c) period 3 and (d) period 4. The error bars indicate standard errors.

Patterns of water extraction changed with depth according to period of observation (Fig. 7.5). Periods 1, 2 and 3, which commenced without water limitation, had similar patterns of water use reaching 50% water extraction at 0.9, 1.2 and 0.8 m depths, while 90% was reached at 2.5, 2.6 and 3 m depths respectively. However, period 4 which commenced without irrigation extracted water deeper in the profile reaching 50 and 90 % water extraction at 2.4 and 3.85 m depths respectively (Fig. 7.5).

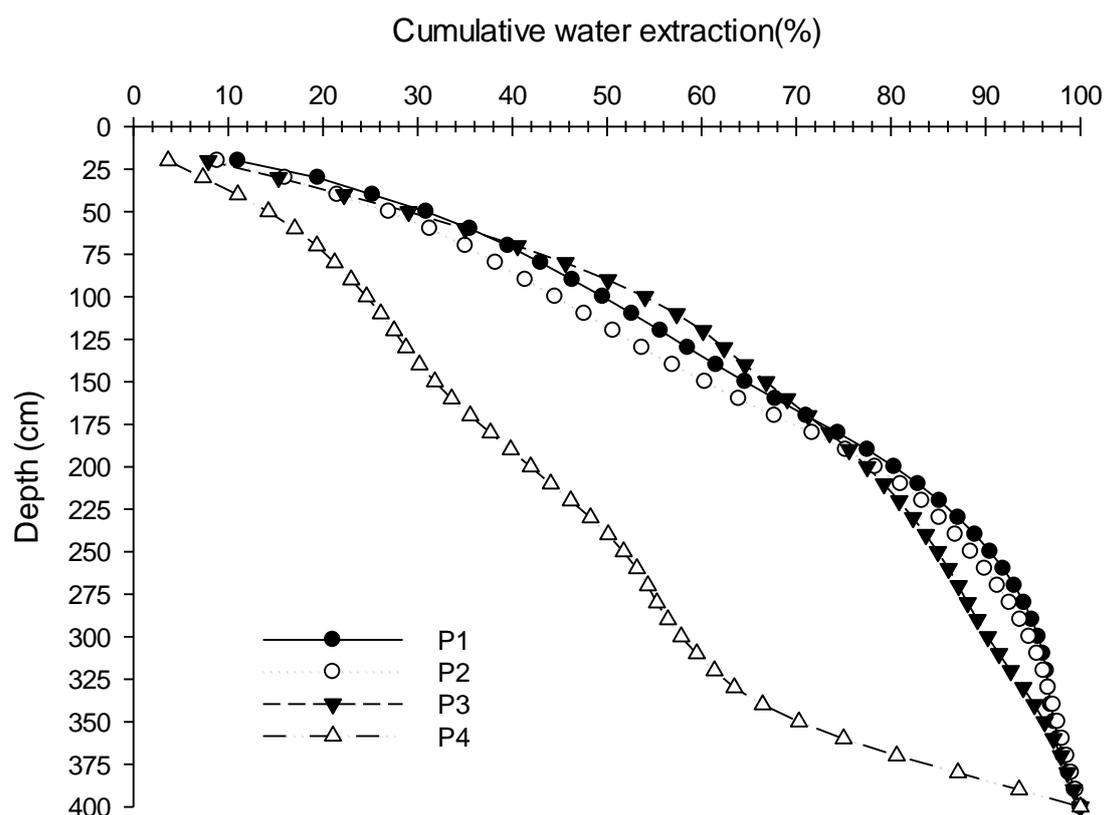


Fig. 7.5: Patterns of water use expressed as cumulative percentage (%) of total water extraction with increment of soil depth during the four periods of study.

7.3.5 Lateral patterns of water extraction (Diviner 2000 data)

Analysis of Diviner 2000 data for all periods also indicated that rate of water extraction (mm d^{-1}) was similar between LD and HD within-row ($P > 0.05$) but varied according to the interaction between lateral distance and depth ($P < 0.001$). In general, greater water extraction occurred in the top soil and decreased with depth forming “S-shaped” patterns (Fig. 7.6). The “S-shaped” patterns was not observed using NMM data, probably as sampling depths by NMM were 0.5 m apart compared to 0.1 m for the capacitance probes. Water extraction varied laterally, with greater water extraction observed near the leucaena row reducing with increment of lateral distance from the tree (Fig. 7.6a, 7.6b and 7.6c). An exception was also observed in P4 as greater and deeper water extraction registered at 3 m distance from leucaena trees (Fig. 7.6d). As expected, P1 and P3 had similar values for water extraction as leucaena plants experienced similar temperature and soil water conditions. Periods 2 and 4 coincided with the growing season of leucaena and greater values of water extraction were expected. Similar to NMM, period 2 registered the highest rate of water extraction, while P4 registered the lowest rate of water use (Fig. 7.6b and 7.6d).

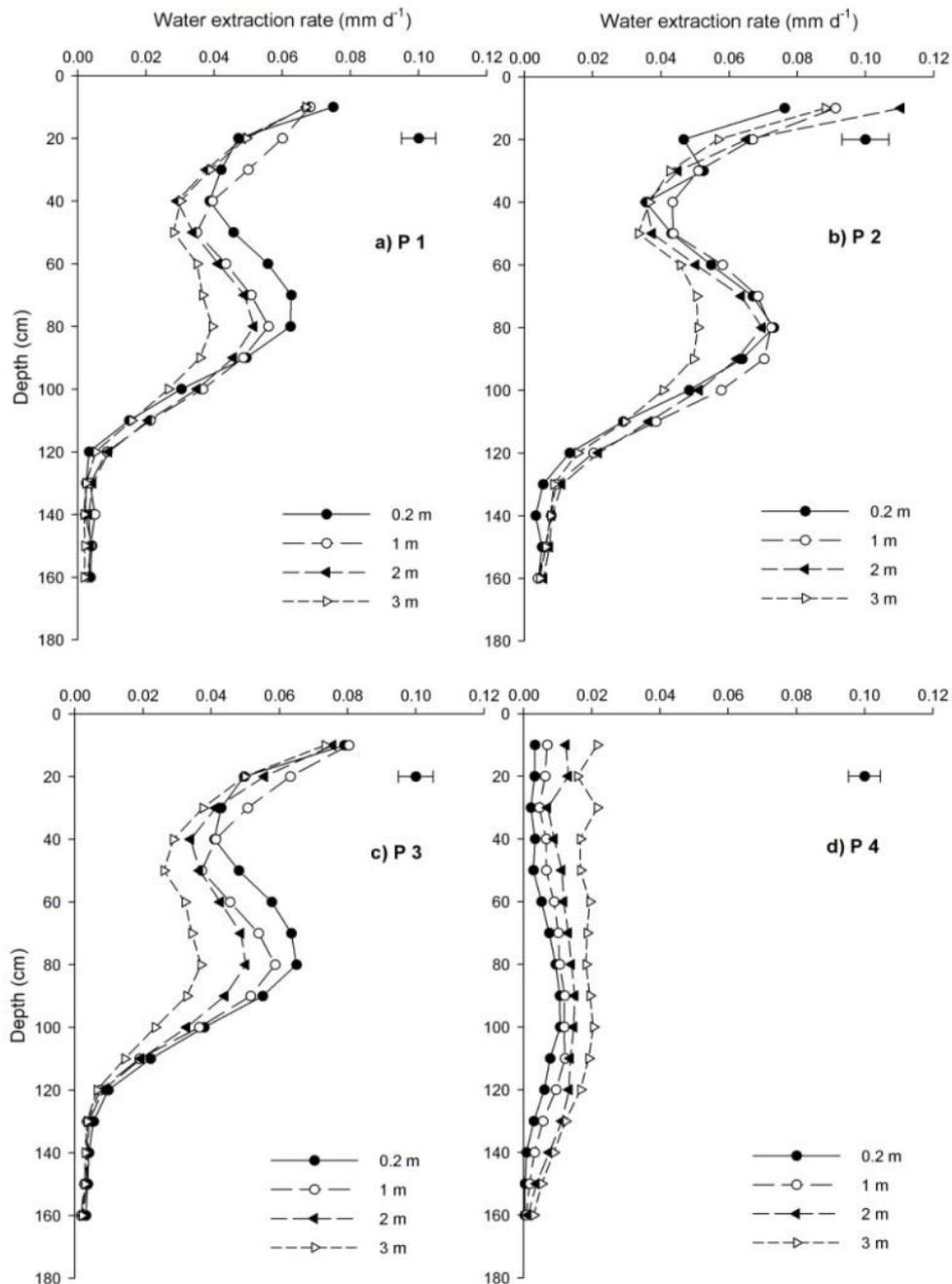


Fig. 7.6: Average water extraction rate measured by Diviner 2000 probes at four distances from leucaena trees (0.2, 1, 2 and 3 m) to 1.6 m depth: (a) period 1, (b) period 2, (c) period 3 and d) period 4. The error bars indicate LSD ($P=0.05$) based on the analysis between four distances.

7.4 Discussion

7.4.1 Effect of within-row density on above-ground biomass

In this experiment, it was hypothesized that increasing plant density within-row spacing of leucaena would lead to greater above-ground biomass yield per unit area. However, while individual tree above-ground biomass of leucaena and leaf area were lower with closer within-row spacing, total above-ground biomass and leaf area expressed as per linear metre of hedgerow was not different in periods 2–4.

Previous studies on leucaena have reported that increasing plant density reduced the numbers of branches, leaf area and stem diameter compared to trees growing at low densities, while total above-ground biomass per unit area increased (Cooksley and Goward, 1988; Budisantoso, 2005; Chotchutima *et al.* 2013). Similar findings were reported by Karim and Savill (1991) in an agroforestry study of the effect of four between-row spacing (2, 4, 6 and 8 m) and 3 within-row spacings (0.25, 0.5 and 1 m) on biomass of *Gliricidia sepium*. They

also found that closer between-row spacing increased biomass production per unit area, while closer within-row spacing reduced biomass production per plant.

It was therefore evident that despite different row spacing, growth of individual trees was able to compensate for lower density given sufficient time. However, when growth resources are limiting, such as PAW at the last harvest period (P3–P4), the effect of drought stress on growth and especially leaf area was evident. After 365 days without rainfall, leucaena plants had LA per linear meter reduced by ~38% compared to harvest in P2. Similar results were reported by Budisantoso (2005), who found that, regardless of density, leaf biomass decreased by 36–50% when trees were exposed to severe drought stress conditions. In addition, Aref (2005) reported approximately 50% reduction in leaf and branch growth of *L. leucocephala* and *Albizia lebbbeck* under water stress conditions. It is well known that drought stress negatively influences leaf growth (Hsiao, 1973).

7.4.2 Effect of within-row density on rooting patterns

The hypothesis that leucaena growing at higher densities within-row would have increased rooting abundance was also not proven. Both, high and low spacing within-rows were characterized by similar deep and lateral spread of roots. Although root sampling was limited to 4 m depth and 3 m distance from the tree rows, trends indicated that leucaena roots extended beyond these limits. Other studies of leucaena-grass pasture in Central Queensland, in a soil without physical restrictions, reported roots of leucaena to 5–6 m depth (Poole, 2003) and 4 m depth (Pachas *et al.* 2016). Greater lateral spread to 3.5 m was observed in leucaena growing without crop competition (Gosh *et al.* 1989). In the Nelder fan experiment (Chapter 6), lateral spread of roots to 5 m was observed when leucaena was grown at very low density without grass competition. However, with increments of density and adding grass competition, lateral spread of leucaena roots was considerably reduced (Chapter 6).

Overall, root abundance of leucaena declined with depth; however there was not greater abundance of fine roots in the top soil (0–1 m) as reported by other authors (Jonsson *et al.* 1988; Dhyani *et al.* 1990; Poole, 2003). In this study, many fine roots were observed between 1–2 m depth in the soil profile. It is hypothesized that rooting patterns of leucaena were affected by the long drying-cycle under the rainout shelter, and this caused fine roots of leucaena to move deeper in the soil profile searching for plant available water which was depleted in the upper soil. Similar results were observed with *Acacia saligna* shrubs grown under different irrigation frequencies in an arid environment (Zegada-Lizarazu *et al.* 2007). They reported that the bulk of roots were found at increasing depth as irrigation frequency decreased.

7.4.3 Effect of within-row density on water extraction

The hypothesis that water extraction would vary with density within-row was also rejected. Water extraction of leucaena growing without water limitation was greatest from the upper soil profile and declined with depth. Similar findings were reported by Budisantoso (2005) where most water was extracted to 0.6–0.8 m depth when there was no water limitation. Eastham *et al.* (1990), working with *Eucalyptus grandis* and *Setaria sphaceolata*, reported that water uptake by trees was mostly confined to the upper soil layer before a period of drought commenced. However, as the water content decreased with time as a consequence of the drought, there was an increased proportion of water extracted from deeper in the soil profile. In the present study, an increased proportion of water extraction in deeper soil was also observed in period 4 after a prolonged dry period. These results are thus in accordance with findings of Budisantoso (2005) with forage tree legumes and Eastham *et al.* (1990) and Dye (1996) with trees.

In contrast, monitoring water extraction patterns of leucaena-grass pasture in a field environment in Central Queensland, Pachas *et al.* (2016) did not observe deep soil water extraction as soil water was depleted in the upper horizons. The probable reason for this was that water uptake was negatively affected by pruning and continuous heavy grazing thus reducing leaf area and water demand.

Changes in the lateral water extraction patterns were also observed according to status of soil water content. In periods without limited plant available water in the upper soil profile, most water was extracted near to

the trunk and decreased with lateral distance from the tree. However, during drought, when most water was depleted near to the leucaena row, an increased proportion of soil water was extracted more than 2 m distance. This became an important source of water as there was no competition for water resources by crops or grasses. It is expected that level of lateral water extraction would be reduced when leucaena is growing and competing with crops or pasture or when narrow leucaena rows limit lateral soil water available. Similar results were reported by Eastham *et al.* (1990) who found that water uptake of eucalyptus was generally lower at 3.25 m than at 1.2 m from trees at medium and low tree densities. Others results were reported by Huth *et al.* (2010) in a study of competition for soil moisture by *Eucalyptus argophloia* windbreak with associated crops. They found that rates of water extraction decreased with distances from the trees.

7.5 Conclusions

The within-row densities of leucaena tested did not differ in patterns of rooting distribution and water uptake. When leucaena was grown without water limitation, most soil water was extracted from the upper soil layer. However, when it was grown in a long drought period, vertical as well as lateral patterns of soil water extraction changed and leucaena was able to extract a greater proportion of water from deeper in the soil profile and further from the hedgerow. Thus leucaena growing at wider spacings within row had great plasticity to generate branches and leaf biomass and was able to capture the same resources as when grown at close within hedgerow spacings.

8 Completion of project objectives

The project objectives and their respective completion / achievement rates were as follows.

- 8.1 *Measured root architecture and soil water extraction patterns of the leucaena and grass components of pasture, including the impact of within-hedgerow leucaena.*

Objective achieved.

- 8.2 *Determine the impact of leucaena hedgerow spacing, and associated competitive interactions, on partitioning of total water use between leucaena and grass.*

Objective achieved

- 8.3 *Determine the ability of leucaena and grass to extract soil water from different depths in the soil profile.*

Objective achieved

- 8.4 *Quantify seasonal changes in leucaena and grass evapotranspiration crop factors.*

Objective achieved but data reported separately via the assistance and modelling work of Dr John Murtagh

- 8.5 *Evaluate the impact of degree of defoliation of leucaena and inter-row grass on rates of recovery of plant water use and forage production.*

Objective achieved for leucaena but was not completed for Rhodes grass

- 8.6 *Provide management recommendations for hedge-row system design and grazing management that will reduce establishment costs and optimise forage productivity for varying levels of soil water availability in representative agroclimatic environments.*

Objective achieved

9 Conclusions/recommendations

The main objective of the study was to investigate the above- and below-ground interactions in relation to water use of leucaena-grass pasture. Previous studies were reviewed and provided evidence that there was limited understanding concerning these interactions and that most previous studies had focused on above-ground interactions only. The principal hypothesis of the study was that there would be strong complementarity in the use of soil water due to minimal overlap of feeder roots. This hypothesis was not supported by the results due to strong below-ground competition for soil water resources in the top 1.5 m of the soil profile where the majority of roots of both species were situated. The results of the study have led to the following conclusions.

9.1 A case study in southern inland Queensland, Australia: competition and complementarity in a leucaena-grass pasture

This initial on-farm monitoring study highlighted that there was minimal spatial and temporal complementarity between leucaena and Rhodes grass for soil water resources. This was contrary to the general agroforestry hypothesis that trees acquired water from different soil strata when grown in association with more shallow-rooted grasses (Schroth, 1999). Although depth of root activity of leucaena and Rhodes grass varied according to seasons, and leucaena roots extracted water from deeper in the soil profile, a high level of competition for water was evident as most of the extraction occurred in the top 1.5 m of the soil profile.

In the second year of the study, characterized by a dry-period, it was expected that water extraction by leucaena would occur deeper in the soil profile. However, root activity was detected in shallower parts of the soil profile probably associated with severe defoliation caused by grazing and pruning. Another important outcome of this experiment was the low level of deep drainage of soil water below 4 m.

This study was valuable as it was the first detailed information of soil water use by a leucaena-grass production system. Also the constant monitoring using EnviroScan technology with sensors located from the surface to 4 m depth, and a sampling interval of 15 minutes, meant that a very large data set was accumulated.

9.2 Effect of defoliation

Results of the defoliation experiment showed that intensity of defoliation directly influenced water uptake of leucaena. Thus, in practical situations, management of grazing/pruning intensities can be used to reduce or even increase water use of leucaena. Surprisingly, light levels of defoliation (25% removal of leaf) stimulated leaf and shoot growth increasing water uptake compared to control plants that were not defoliated. However, cutting to 1 m height reduced 79% of cumulative water extraction compared to defoliation of $\leq 75\%$ of leaf.

9.3 Effect of plant density

Above-ground interactions

The results from the Nelder fan experiment confirmed that density of leucaena had a strong effect on intra and interspecific competition between leucaena and Rhodes grass. Contrary to expectation, the combination of leucaena and Rhodes grass did not improve system productivity (in terms of above-ground biomass) due to the strong interspecific competition for light and water resources. Strong grass competition reduced leucaena yield per tree by 50–70% when it was grown at low densities between 100 to 4,100 trees ha⁻¹ mainly due to competition for soil water. However, leucaena grown at high density reduced Rhodes grass yield to zero due to high light interception and competition for water.

Above-ground leucaena yield per unit area increased with tree density, although trees were smaller with a reduction in cross-sectional basal area of stems, number of stems and leaf area per tree.

Tree density strongly influenced the relative proportions of above-ground biomass of leucaena and grass with approximately 10, 50 and 90% of total yield being leucaena achieved at tree densities equivalent to 100, 1,500–2,000 and 7,000–8,000 trees ha⁻¹ respectively.

Above-ground biomass of leucaena and Rhodes grass was also influenced by age of the stand. Rhodes grass grew vigorously during the first year, while during the second year, the combined effect of lower soil nitrogen available and increasing competition for light and water reduced Rhodes grass yield.

Plant nutrition status of leucaena at different densities was not influenced by inter- and intraspecific competition with an exception of phosphorous which was at marginal levels regardless of tree density and grass competition.

Below-ground interactions

It was concluded that a disparity in root abundance of leucaena and Rhodes grass led to the low degree of complementarity. While leucaena roots reached deeper in the soil profile, there was strong competition with Rhodes grass roots as most of the roots from both species, and therefore water extraction, were observed in the top ~1.5 m of the soil profile.

Rhodes grass had root abundance between 8-10 times greater than leucaena which allowed it to compete more effectively for water resources and limit the lateral spread of leucaena roots.

In the rainout shelter experiment which compared within-row leucaena densities (1 trees m⁻¹ and 10 trees m⁻¹) there was no effect on soil water use and rooting patterns. Leucaena plants grown at wider within-row spacings had the plasticity to generate increased branch and leaf biomass and thus capture the same resources as when grown at closer within-row spacings.

Without water limitation, patterns of water uptake were characterized by withdrawal of water in the upper soil profile (0-1.5 m depth). However, after long dry periods in the rainout shelter, there was evidence that leucaena had the ability to extract a higher proportion of water from deeper in the soil profile.

9.4 Limitations of the experiments

It is important to note that outcomes of Nelder fan experiment cannot be extrapolated directly to densities in commercial leucaena-grass systems as recommendations may differ according to planting configuration (single or twin-row hedgerows), soil restrictions, grazing management or grass combination. The Nelder fan design has the limitation that as tree density increases, the hedgerow spacings become narrow. In commercial leucaena-grass pastures, tree densities can vary by keeping hedgerow spacings fixed and increasing densities within-rows or using twin hedgerows. For instance, the biomass yield of Rhodes grass measured at high leucaena density (>8,618 trees ha⁻¹) was influenced by shade in the Nelder fan configuration. Greater biomass yield of grass would be obtained at similar tree densities by planting trees using closer within-row spacing. Another limitation might be expected when competition for water resources is exacerbated by soil physical restrictions. In this case roots will be concentrated in the upper soil profile. However, the advantages of the Nelder fan experiment was the ability to study the effect of 10 tree densities with and without grass competition in a compact and cost effective manner.

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10 Key messages

In general, very little complementarity was observed between grass and leucaena due to the intense competition for soil water occurring in the top 1.5 meter of the soil profile.

Plant water availability should be considered as the main determining factor in choice of hedgerow configuration. For instance, some graziers plant wider leucaena hedgerows (>10 m apart) arguing that leucaena plants will have better access to soil moisture and will perform better in dryland areas. However, this project has demonstrated that this practice will promote grass growth at the expense of leucaena production.

Key messages for industry include:

- Leucaena can extract soil water to 3 m depth and deeper in dry times demonstrating its drought tolerance. Very little deep drainage of soil water will occur below the leucaena root zone and only following large rainfall events in those rare circumstances when the profile was already full to 4m.
- Tropical grass outcompetes the leucaena hedgerows for soil moisture. Thus grass competition should be eliminated during leucaena establishment as grass competition for soil moisture reduces above-ground growth and significantly reduces leucaena rooting exploration, water uptake and biomass yield. The importance of fallowing paddocks to store a profile of soil moisture prior to leucaena establishment and then to maintain complete weed and grass control until leucaena is over 2m tall is of utmost importance.
- Wide hedgerow spacing (> 8 m) is not recommended as this configurations heightens the competitive advantage of the grass compartment of the pasture, suppressing the leucaena production.
- In areas of high rainfall, or under irrigation, wider row spacing <6-8m can be recommended to increase the amount of inter-row grass available without reducing leucaena growth within the hedgerows. This would have the advantage of permitting higher stocking rates, and better height management of leucaena hedgerows which are prone to excessive growth in high moisture environments.
- Increasing the leucaena population in the pasture by using narrower row spacings, will reduce grass competition for soil water and promote higher yields of leucaena component of the pasture.
- Within hedgerow populations of the leucaena plants at densities of 1-10 plants per metre of row, did not affect leucaena rooting pattern or extent of soil water extraction. Further study of even lower within-hedgerow density is required to determine the critical plant density below which yield is compromised.
- Leucaena defoliation management can affect water use, reducing almost to nil under severe defoliation, while mild pruning (<25%) can stimulate water uptake of leucaena.
- Profitability of leucaena grass pasture depends upon successful establishment of the leucaena hedgerows, their sustained production of high-quality located forage for more than 20 years, to maximise animal nutrition and beef production.

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12 Appendix

The publication submitted to international journals during the project was:

Pachas ANA, Shelton HM, Lambrides CJ, Dalzell SA, MacFarlane DC, Murtagh GJ (2016) Water use, root activity and deep drainage within a perennial legume-grass pasture: A case study in southern inland Queensland, Australia. *Tropical Grassland-Forrajes Tropicales* 4:129-138. DOI:

[http://dx.doi.org/10.17138/TGFT\(4\)129-138](http://dx.doi.org/10.17138/TGFT(4)129-138)

The publication submitted to international congresses and conferences were:

Pachas ANA, Shelton HM, Lambrides CJ, Dalzell SA, Murtagh GJ (2015) The effect of tree densities on the biomass of *Leucaena leucocephala* and *Chloris gayana* using a Nelder fan design. Proceeding XXIII International Grassland Congress, November 20-24, 2015, New Delhi, India (Appendix 1).

Pachas ANA, Shelton HM, Lambrides CJ, Dalzell SA, Murtagh GJ (2015) The effect of plant density on water use and root distribution in *Leucaena leucocephala* under a rainout shelter. Proceedings ISSR 9: Roots Down Under. From 6 to 9 October, 2015, Canberra, Australia (Appendix 2).

Pachas ANA, Shelton HM, Lambrides CJ, Dalzell SA, Murtagh GJ (2015) The effect of tree density on biomass production of *Leucaena leucocephala* and *Chloris gayana* using a Nelder fan design. Proceedings of the Northern beef Research update conference, 15-18 August, Rockhampton, Australia. Page: 156 (Appendix 3).

12.1 Appendix 1

The effect of tree densities on the interaction of *Leucaena leucocephala* and *Chloris gayana* using a Nelder fan design

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Key words: agroforestry, competition, legume, yield Rhodes grass

Introduction

Leucaena leucocephala (leucaena)-grass pastures are widely used for ruminant feeding in tropical and subtropical regions. In Australia, over 200,000 ha of leucaena grass pasture have been planted with more plantings expected as it is recognised as the most productive, profitable and sustainable feeding system (Shelton and Dalzell, 2007). Planting densities and planting configurations for the leucaena component vary, ranging from single or double leucaena hedgerows 3 to 12 m apart (Radrizzani *et al.*, 2010).

There is little information about how tree/grass planting configurations and resulting inter- and intraspecific competition affect above and below-ground interactions. We hypothesise that individual leucaena tree biomass will be inversely related to leucaena tree density, with greatest competition at low density, while medium to high leucaena densities will reduce grass biomass production.

Materials and Methods

Experimental Site

The experiment was conducted at the Gatton research farm (27.54°S, 152.34°E) of the University of Queensland, Australia. The soil was a deep (3 m) Black/Brown Haplustoll. The climate is subtropical, with an average rainfall of 798 mm per annum and average maximum and minimum temperatures of 31.6 and 19.3°C in the hottest month (January) and 20.7 and 6.2°C in the coolest month (July) (Bureau of Meteorology, 2015).

Leucaena leucocephala (Lam.) de Wit ssp. *glabrata* (Rose) Zarate cv. Tarramba was planted in a Nelder fan design on 27th November 2013. Twelve concentric rings of trees with radii of 0.6, 0.9, 1.3, 1.9, 2.8, 4.0, 5.9, 8.5, 12.3, 17.9, 25.9 and 37.6 m were planted. Each ring contained 16 trees planted at equi-distance around the circumference, giving a range of tree densities: 100, 210, 442, 928, 1,951, 4,100, 8,618, 18,112, 38,065 and 80,000 trees/ha. *Chloris gayana* (Kunth.) cv. Finecut was sown in March 2014 in two quarters of the Nelder fan. The total area occupied by the experiment was 0.47 ha.

Measurements

Above ground biomass was harvested 3 times over 231 and 252 day growth periods for Rhodes grass and leucaena respectively during 2014 and 2015. For each leucaena density, total biomass of 8 trees was measured (4 with Rhodes grass and 4 without grass). The leucaena was harvested to a height of 1 m. The basal diameter of several stems/tree was measured with callipers and total biomass estimated using a robust regression relationship between biomass (g DM/stem) and cross sectional area of the cut stem (cm²). For each harvest, a calibration equation was prepared based on the measurements of 25-45 stems. Regression coefficients (R²) of 0.91, 0.99 and 0.99 corresponded to harvests on 10/10/14, 9/12/14 and 21/01/15 respectively. The above-ground biomass of Rhodes grass was estimated using BOTANAL sampling procedures (Tothill *et al.* 1978). Regression calibration equations linking biomass to visual yield score had R² values of 0.92, 0.97 and 0.99 for harvests on 7/10/14, 19/11/14 and 20/01/15 respectively.

Data analysis

Scatters plots of accumulated leucaena above-ground biomass (kg DM/tree and kg DM/ha) and Rhodes grass biomass were made against leucaena density leucaena (trees/ha). Following the methodology of Ritchie (1997), tree density was log transformed and the data subjected to non-linear and linear regression. The statistical software used was Minitab (version 16.2.4, MiniTab Inc, State Collage, PA) and SigmaPlot (version 12, Systat, San Jose, CA).

Results and discussion

As anticipated, leucaena tree density strongly influenced individual tree biomass (kg DM/tree) and total leucaena biomass (kg DM/ha) ($p < 0.001$). Individual leucaena tree yield was negatively related to the log of tree density (R² = 0.99). Maximum biomass (8 kg DM/tree) was reached at 100 trees/ha without grass competition, and was reduced by 62 % with grass competition (Fig. 1). The individual biomass of leucaena trees was reduced with increasing density due to intraspecific competition reaching 0.2 kg DM/tree at 38,065 and 80,000 trees/ha. There was no effect of Rhodes grass competition on leucaena biomass at tree densities above 10,000 trees/ha due to poor vigour of the grass sward.

Individual tree biomass was reduced due to interspecific competition with Rhodes grass at tree densities of 100 to 928 trees/ha. At densities >1951 trees/ha grass growth was negatively affected by competition for light and water resources, and had negligible impact on individual tree yield.

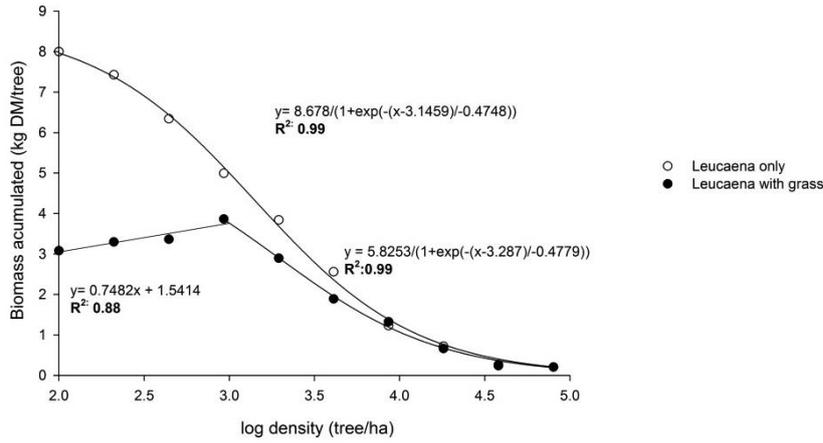


Fig. 1: Relationship between the individual cumulative tree biomass (kg DM/tree) over a 252 day period and log of leucaena tree density (trees/ha).

Accordingly, total biomass/ha of leucaena was positively related to the log of leucaena density ($R^2 = 0.97$) regardless of grass competition, reaching 16,540 kg DM/ha at the highest leucaena density of 80,000 trees/ha (Fig. 2). In contrast, the yield of Rhodes grass was linearly and inversely correlated with the log of tree density ($R^2 = 0.97$). There was no grass growth at densities $\geq 11,120$ trees/ha. At low tree densities, the reduced leucaena yield due to grass competition had a minor impact on total yield. The grass component constituted 97% (10,050 kg DM/ha), 50% (4,952 kg DM/ha) and 5% (609 kg DM/ha) of total biomass at tree densities of 100, 1578 and 8618 trees/ha respectively. Meanwhile, the leucaena component varied from 36% (3583 kg DM/ha) to 77% (7.742 kg DM/ha) at 442 and 4100 trees/ha respectively (Fig. 2).

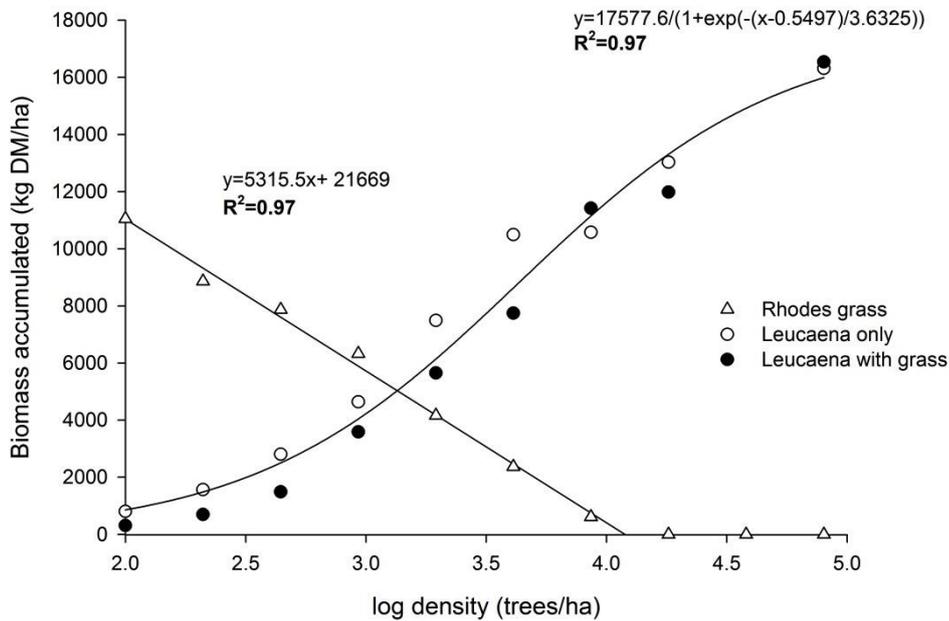


Fig. 2: Relationship between cumulative biomass (kg DM/ha) of Rhodes grass (231 days) and leucaena (252 days) and the log of leucaena tree density (trees/ha).

The Nelder fan design was a useful approach to evaluate the effect leucaena density on intra-tree and inter-specific competition between leucaena and Rhodes grass. However, the outcome of competition may be different depending upon the planting configuration of tree and grass species.

Configurations of leucaena trees comprising close tree spacing within leucaena hedgerows combined with wide alleys between rows will enhance the light interception by the inter-row grass.

Conclusion

Leucaena and Rhodes grass can be successfully grown together to provide both high quantity and high quality forage for animal production. The relative yield contributions of the two components will be determined by the density of leucaena trees given equidistant planting configurations. The outcome of interspecific competition may be different if leucaena is planted in high density hedgerow configurations with grass grown between the rows.

Further studies focusing on below-ground competition such as root architecture and patterns of water uptake are ongoing to a better understanding of leucaena-grass systems.

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12.2 Appendix 2

The effect of plant density on water use and root distribution in *Leucaena leucocephala* under a rainout shelter.

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Introduction

Leucaena leucocephala (Lam.) de Wit (leucaena)-grass pastures are recognized as a productive, profitable and sustainable feeding system for beef cattle production in Queensland, Australia (Shelton and Dalzell, 2007). The limited understanding of distribution, dynamics and the complex interactions that occur at the root level between trees and grass is a major obstacle to enhanced design and management of these pastures (Rao *et al.* 2004). This study aimed to examine the differences in root distribution and water extraction of leucaena established at two hedge row densities under a rainout shelter. We hypothesized that leucaena planted at low density would have a shallower root system, have a smaller canopy per unit area and therefore would extract less soil water.

Methods

The experiment was conducted under rainout shelters at the Gatton research farm (27.54°S, 152.34°E) of the University of Queensland, Australia. *Leucaena leucocephala* ssp. *glabrata* (Rose) Zarate cv. Tarramba was planted on 29th November 2013. Single row plots were arranged in a randomized complete block design with 4 replications and 2 within-row densities: a) low density (1 plant every 100 cm) and b) high density (1 plant every 10 cm). No grass was established in the inter-row and plots were kept weed free. In each plot, three 400 cm-deep Neutron Moisture Meter (NMM, model CPN 503 DR Campbell Pacific Nuclear International Inc., USA), access tubes were installed at 20, 50 and 100 cm perpendicular to the row to monitor soil water content. The experiment was maintained at (or close to) field capacity until the 19th March 2014 after which water was withheld. Soil water extraction was monitored every 14 days for the next 112 days to 9th July 2014. Above ground biomass per tree was determined by destructive harvest and the leaf area per tree was measured with a LI-COR 3200 (Li-Cor, Inc., Lincoln, NE) on 14th July 2014.

Results and Discussion

Preliminary results (Table 1) show that leucaena hedge row density had an effect ($P < 0.05$) on tree biomass yield and leaf area. Our hypothesis was rejected because, a tenfold increase in density led to higher biomass yield, but similar total water use.

Table 1: Effect leucaena hedge row density on: above ground biomass, leaf area, rooting depth and water extraction.

Variable	Treatment		S.E
	Low Density	High Density	
Individual tree biomass (kg DM/tree)	0.42 a	0.19 b	±0.12
Total biomass (kg DM/lineal metre)	0.42 a	1.87 b	±1.5
Individual tree leaf area (cm ² /tree)	14809 a	8767 b	±267.4
Total tree leaf area (cm ² /lineal metre)	14809 a	87659 b	±626.3
Maximum active root depth registered (cm)	330 a	355 a	±20.7
Average total water extracted (mm)	139.9 a	150.4 a	±13.4
Average daily water use (mm/day)	1.23 a	1.33 a	±0.12

* Different letters within a row indicate tree density means are significantly different at $P < 0.05$.

Conclusion

Leucaena hedge row density can affect tree biomass yield and leaf area. However, soil water extraction by leucaena was not affected by hedgerow density. Further investigation of lateral root soil exploration will be undertaken in this study.

Rao MR, Schroth R, Williams SE, Namirembe S, Schaller M, Wilson J (2004) Managing Below-ground Interactions in Agroecosystems. In van Noordwijk, G. Cadisch, and C. Ong (eds.) "Below-ground Interactions in Tropical Agroecosystems: concepts and models with multiple plant components" CABI Wallingford, UK, pp. 309-328
Shelton, M., Dalzell S. 2007. Production, economic and environmental benefits of leucaena pastures. *Tropical Grasslands* 41: 174–190.

12.3 Appendix 3

The effect of tree density on biomass production of *Leucaena leucocephala* and *Chloris gayana* using a Nelder fan design

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Introduction

Leucaena leucocephala (leucaena)-grass pastures are widely used for beef production in tropical and subtropical Queensland, where it is recognised as a productive, profitable and sustainable feeding system (Shelton and Dalzell 2007). There is limited information regarding how plant density of leucaena affects competition between tree and grass components of the pasture.

Methods

A Nelder fan design with 10 different leucaena densities (from 100 to 80,000 trees ha⁻¹) growing with and without Rhodes grass (*Chloris gayana*) was established at Gatton, Queensland in November 2013 (Pachas *et al.*, 2015). The above-ground biomass was determined 4 times within 386 and 412 day growth periods for Rhodes grass and leucaena, respectively, during 2014 and 2015.

Results and Discussion

The individual yield of leucaena plants was negatively affected by increasing leucaena plant density expressed as log₁₀ (trees/ha). Maximum individual yield (23.7 kg DM tree⁻¹ year⁻¹) was obtained at a density equivalent to 100 trees ha⁻¹ without grass competition and was reduced by 68% with grass competition. However, total leucaena and Rhodes grass biomass expressed as kg DM ha⁻¹ year⁻¹ was positively correlated to log₁₀ of tree density (R²=0.99) reaching 32,800 kg DM ha⁻¹ year⁻¹ at the highest density tested (80,000 trees ha⁻¹). The yield of Rhodes grass was negatively correlated with increasing leucaena plant density (R²=0.99). Maximum grass yield of 24,260 kg DM ha⁻¹ year⁻¹ without tree competition declined to 1,420 kg DM ha⁻¹ year⁻¹ at a leucaena plant density of 8,618 trees ha⁻¹. The contribution of leucaena and grass to total forage production varied with tree density; leucaena forage constituted 10%, 50% and 90% of total edible biomass at tree densities of 210, 1,457 and 8,618 trees ha⁻¹ respectively.

Conclusions

Leucaena biomass yield was significantly reduced (>50%) by grass competition at low leucaena plant densities (100 to 4,100 trees ha⁻¹). At high leucaena plant densities (>5,000 trees/ha), double the amount of leucaena biomass forage was produced but grass growth was minimal. At high leucaena plant densities, grass/roughage would need to be provided from adjoining paddocks or as a supplement to deliver a balanced diet to grazing cattle.

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