Water use and bioenergy potential of subtropical *Poaceae* species as second generation field crops

by

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ABSTRACT

First generation (edible) crops used for bioenergy production are generally not economically feasible due to the fact that they rarely offer significantly more energy at the end of the processing chain as compared to the energy required throughout all the production steps. South Africa, being a water scarce country will rely heavily on bioenergy crops with high water use efficiency in conjunction with high overall energy production to effectively produce sustainable and renewable forms of energy, and thus this study also investigated the responses of different bioenergy crops to water regimes. Knowledge is lacking in the areas of water use, water use efficiency, potential energy yield and management of non-edible (second generation) bioenergy crops under South African climatic conditions. The aim of this study was to determine how biomass production and corresponding calorific values are affected by different water regimes and harvesting intervals. A two-factorial split-plot randomised block design field experiment with three water regimes, eight *Poaceae* species and three replicates (plot size 5.5 m x 6 m) was conducted. Three regimes of increasingly available soil water were applied, namely: dryland (T1), two-weekly (T2) and weekly (T3) irrigation according to soil water content measurements. Harvesting was done monthly for three months in successive sections of each plot and this cycle was repeated three times (summer (C1), autumn (C2) and spring (C3) cycles). The following *Poaceae* species were analysed in the present trial: *Panicum maximum, Pennisetum purpureum*, *Miscanthus giganteus*, *Chrysopogon zizanoides, Hyparrhenia tamba, Brachiaria brizantha*, *Sorghum bicolor* (sweet sorghum) and *Sorghum bicolor* (grain sorghum), with sweet sorghum as the control species. It was noted that more frequent harvest intervals within the same time period compared to a single final harvest generally did not produce greater biomass yields. The average annual water to energy production efficiency (WEPE) values for each species across all water treatments are listed in descending order: *H. tamba* (S6 – 601 MJ ha^{-1} mm^{-1}), *P. maximum* (S1 – 549 MJ ha^{-1} mm^{-1}), *P. purpureum* (S2 – 502 MJ ha^{-1} mm^{-1}), *B. brizantha* (S7 – 477 MJ ha^{-1} mm^{-1}), *C. zizanoides* (S5 – 454 MJ ha^{-1} mm^{-1}), *S. bicolor* (S8 – 309 MJ ha^{-1} mm^{-1}), *S. bicolor* (S9 – 185 MJ ha^{-1} mm^{-1}) and *M. giganteus* (S4 – 113 MJ ha^{-1} mm^{-1}). The greatest average WEPE across all species was produced at T2 (415 MJ ha^{-1} mm^{-1}), followed by T1 (398 MJ ha^{-1} mm^{-1}) and T3 (384 MJ ha^{-1} mm^{-1}). From preliminary data it can be concluded that *H. tamba*, *P. maximum* and *P. purpureum* are the three most promising *Poaceae* species for bioenergy production under South African climatic conditions. Also, *P. maximum* and *B. brizantha* are likely better alternatives for annual rotation crops as compared to the two sorghum varieties. Lastly, indigenous species convincingly outperformed exotic species.

DECLARATION

I Heinrich Cloete, student of the Faculty of Natural and Agricultural Sciences of the University of Pretoria, aware of my responsibility of the penal law, declare and certify with my signature that my dissertation entitled **Water use and bioenergy potential of subtropical *Poaceae* species as second generation field crops**, is entirely the result of my own work. I have faithfully and accurately cited all my sources, including books, journals, handouts and unpublished manuscripts, as well as any other media, such as the Internet, letters or significant personal communication.

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LIST OF ABBREVIATIONS

CV	Calorific value
CO_2	Carbon Dioxide
C1	Cycle 1 (1 Dec 2014 – 28 Feb 2015)
C2	Cycle 2 (1 Mar 2015 – 31 May 2015)
C3	Cycle 3 (1 Sep 2015 – 30 Nov 2015)
DM %	Dry matter percentage
DMY	Dry matter yield
EISA	Energy Independence and Security Act
EY	Energy yield
FMY	Fresh matter yield
IEA	International Energy Agency
LAI	Leaf area index
Mw	Megawatt
NO_x	Nitrogen oxides
PAR	Photosynthetically active radiation
S	Species
S1	*Panicum maximum* (Guinea grass)
S2	*Pennisteum purpureum* (Napier)
S4	*Miscanthus giganteus* (Miscanthus)
S5	*Chrysopogon zizanoides* (Vetiver)
S6	*Hyparrhenia tamba* (Blue thatch grass)
S7	*Brachiaria brizantha* (Brazilian grass)
S8	*Sorghum bicolor* (Sweet sorghum)
S9	*Sorghum bicolor* (Grain sorghum)
SOM	Soil Organic Matter
SO_x	Sulphur oxides

T	Water treatments
T1	Treatment 1 (dryland water treatment)
T2	Treatment 2 (15 mm irrigation every second week depending on soil water content)
T3	Treatment 3 (15 mm irrigation every week depending on soil water content)
WEPE	Water to energy production efficiency
WUE	Water use efficiency

LIST OF UNITS

^oC	Degrees Celsius
GJ ha^{-1}	Gigajoules per hecatre
GJ ha^{-1} yr^{-1}	Gigajoules per hecatre per year
Gt	Gigatons
l ha^{-1} yr^{-1}	Liters per hectare per year
kg ha^{-1}	Kilograms per hectare
kg ha^{-1} mm^{-1}	Kilograms per hectare per millimetre
kg ha^{-1} yr^{-1}	Kilograms per hectare per year
m^2 m^{-2}	Meter squared per meter squared
MJ ha^{-1}	Megajoules per hectare
MJ ha^{-1} mm^{-1} yr^{-1}	Megajoules per hecatre per millimeter per year
MJ kg^{-1}	Megajoules per kilogram
tons DM ha^{-1} yr^{-1}	Tons dry matter per hectare per year
tons FM ha^{-1} yr^{-1}	Tons fresh matter per hectare per year
tons ha^{-1}	Tons per hectare
tons ha^{-1} yr^{-1}	Tons per hectare per year

CHAPTER 1

GENERAL INTRODUCTION

Reliance on any single energy production system usually becomes problematic when that system is exploited for too long and too intensely. Fossil fuels are a great example of just such a system. Cheap, bountiful sources of energy led humanity down a road of no return. Luxuries became norms and feats which where once impossible were now being undertaken everyday. No time was wasted in seizing these opportunities and soon after the discovery of fossil fuels humanity entered the industrial revolution (Jones 2010). Little thought was given to the effects of using these fuels and especially in the quantities that they were now being consumed. By the time that the populace realized it, the side-effects of fossil fuel energy were in full swing. Clean air became filled with noxious smoke, nitrates and sulphites (Streets and Waldhoff 2000), rain which cleansed the earth and quenched a global thirst became acid rain (Likens et al. 1979), the soil which was depended on to sustain life was mined and left barren, and the earth was enveloped in a blanket of gasses which drastically changed climatic patterns (Nordhaus 1991). To make matters worse, civilization uprooted natural buffers and habitats in order to search for that for which they had developed such a ravenous appetite. The addiction became worse and more seemed to never be enough. Cities expanded, economies grew and soon enough the majority of the global population wanted a taste of the extravagant life.

The foundation upon which these new and lustrous empires were built found its strength in the seemingly endless supply of low-cost energy. Then, accompanied by the imminent reality of diminishing reserves, the threat of that strength failing the human race shook these foundations violently enough for humanity to stop and take note. At the rate that society was consuming these fuels, some estimations predicted that by 2042 (Shafiee and Topal 2009), reserves which had been built up over millennia would be completely exhausted. This vast amount of available fuel was the reaction energy required to sky-rocket the earth's carrying capacity from roughly one billion people prior to the fossil fuel discovery to a global population of over seven billion today (Kunzig 2011). It was at this time that the future reality dawned upon humanity: fossil fuels are a finite energy source and in the long run would become all the scarcer and more expensive to produce. Circumstances would regrettably worsen further by supply security now becoming less and less guaranteed. Then, the unforeseen delivered a final blow. An increase of 2 ^{o}C in average global temperature from before the use of fossil fuels would cause a level of global warming classified as "dangerous". Therefore, to remain below this threshold, only about a third of the remaining fossil fuels could be consumed and thus emitted into the atmosphere (McGlade and Ekins 2015).

The urgent need for alternative energy sources proved to be a blessing in disguise. The opportunities now presented themselves for humanity to halt as well as attempt to remedy the destruction which was being caused to Mother Nature. Alternative energy sources were created and deployed to address the mammoth task of doing away with fossil fuel dependence. Soon, components such as hydro, geothermal, wind, solar and bioenergy formed the pillars of a mindset now focused on renewable and sustainable energy. These sources are all unique and indispensable in their own right. They add to the dynamics and variety necessary to steer away from the mistake humans made once before, when reliance was placed on a sole source of energy to power modern lifestyles. This task, however, proved to be much greater than anticipated and in 2014 only 11% of the world's energy consumption was met by renewable energy sources (Matzenberger et al. 2015). The battle was still being lost but strategies and technologies, however, were constantly being improved. It quickly became evident that optimum efficiency and exploitation of alternative energy sources would be required to replace the quantity of conventional energy which was consumed on a daily basis.

Through this study there is a desire to improve on the bioenergy front and address relevant areas where essential information is lacking. By means of this approach there was a hope that this vital information would be attained, and in so doing lay several more stepping stones towards fossil fuel independence. For the purpose of this study the crude energy potential of selected subtropical *Poaceae* species as viable candidates for bioenergy production was established with regards to several parameters crucial to proficient and sustainable bioenergy production. The word "crude" energy potential was used because from an energy perspective only the calorific values (CV) of the different species were assessed. This gave accurate values of the energy content from an unprocessed point of view but not necessarily accurate values for that of liquid fuels or biogas, for instance. However, the assumption was made that to a fair degree calorific values would be directly proportionate to the energy density of the processed product.

The energy "concentration" of biomass is not the only parameter that needs to be considered. An ideal needs to be achieved between three main pillars: CV, biomass yield and water use efficiency (WUE). All three of these parameters were incorporated in this study in order to weigh up some of the most crucial aspects surrounding the feasibility of applying these species to bioenergy production. Fresh matter yield, seasonal water use and leaf area index were included as secondary parameters to the three main aspects already mentioned.

The aims of this study were to determine:

1. The water use efficiency, as well as seasonal water use of eight different *Poaceae* species subjected to three different water regimes.
2. How different water regimes will affect biomass production and corresponding calorific values.
3. How a single harvest compares to multiple harvests within the same time frame, with respect to biomass yield.
4. The crude energy production versus total water consumption.

CHAPTER 2

LITERATURE REVIEW

Energy production and all its different facets make for a very vast topic of discussion. The purpose of this literature review is thus to dip into that wealth of information and extract relevant data which might put the present study into perspective.

2.1 Fossil fuels

The discovery and unearthing of conventional energy appeared to have exposed a seemingly endless supply of cheap coal, crude oil and natural gas (Gutberlet 2012). As time passed, the grand illusion regrettably began to erode away as the truth revealed that these energy resources were unfortunately finite. However, humanity kept tapping into these assets at an alarming rate (Andres et al. 1999), not too deterred by increased atmospheric carbon dioxide (CO_2) concentrations (Marland and Rotty 1984) or the fact that this energy supply would eventually run out (Crocker and Andrews 2010).

Life before 1860, as it was known in the pre-industrial era (Andres et al. 1999), had undergone a drastic change and before anything could be done to prevent it, humanity had developed a lifestyle which was almost completely dependent on fossil fuels (Council 2007). Global energy consumption increased year after year and reserves kept declining (Owen et al. 2010), but the worst news was yet to come. The world's conventional energy budget would not be able to rely fully on the fuels which still remained beneath the surface of the earth. The general consensus among energy-policy makers is that the threshold for dangerous climate change is at 2 ^{o}C above the average global "pre-industrial boom" temperature (McGlade and Ekins 2015). According to NASA, the average global temperature has risen by 0.8 ^{o}C from 12.9 ^{o}C since 1880. If more than a 50% chance is to exist for the earth to stay below this threshold throughout the twenty first century, then future CO_2 emissions should not exceed a total of 1 100 gigatons (Gt) of CO_2 between 2011 and 2050 (Meinshausen et al. 2009). However, three times as much CO_2 would be released if all proven remaining fossil fuels were to be consumed by 2050 (Meinshausen et al. 2009). Obviously, many different combinations of coal, oil and gas consumption could be configured in order to stay on par with the required environmental restrictions. According to an assessment model used by McGlade and Ekins (2015), an example of such a configuration for the remaining proven reserves would allow for only 67% of oil, 50% of gas and 20% of coal to be used by 2050 if the 2 ^{o}C limit is to be kept. Therefore, the deadline by which sufficient sustainable alternatives have to be implemented has in reality been drastically brought forward. Excessive expenditure on additional exploration in the search of fossil fuel resources should be severely opposed and rather redirected towards finding or refining sustainable alternative energy sources. Failing to do so will only lead to even greater expenditure of resources, energy and money on a well which has already begun to dry (Owen et al. 2010). Moreover, it will delay the adoption of a mind-set change and incorporation of systems which will steer humanity towards renewable and sustainable energy with the necessary haste.

Table 2.1 depicts the very unsettling reality of fossil fuel configurations for different countries, which can altogether be depleted by 2050 if the 2 ^{o}C threshold is not to be exceeded. In this case, "conventional" oil has a density lower than that of water and "unconventional" oil refers to the remaining, heavier type oils. Conventional gas refers to the majority of different natural gas types, with the exeption of three unconventional sources, namely; gas found within relatively impermeable rock, methane adsorbed in the solid coal matrix, and gas found in fine-grained shale. For coal, an energy density in excess of 16.5 MJ kg^{-1} is considered hard coal, whereas coal with an energy density lower than this is classified as lignite (Owen et al. 2010). Unburnable resources refer to resources which should be kept in the ground and never be extracted or combusted in order to restrict global warming and environmental destruction (McGlade and Ekins 2015).

Table 2.1: Regional distribution of resources "unburnable" before 2050 under the 2 ^{o}C scenario (adapted from McGlade and Ekins 2015).

	Conven oil		**Unconven oil**		**Conven gas**		**Unconven gas**		**Hard coal**		**Lignite**	
Country or region	**Gb**	**%**	**Gb**	**%**	**Tcm**	**%**	**Tcm**	**%**	**Gt**	**%**	**Gt**	**%**
Africa	141	50	70	100	28	61	35	100	42	94	2.8	56
Canada	43	72	633	99	3.6	73	18	71	34	98	39	97
China and India	54	60	110	100	8	80	35	88	1003	93	106	88
Former Soviet Union Countries	201	54	360	100	63	67	27	89	576	99	480	98
Central and South America	198	55	447	99	23	76	51	92	21	85	6.3	63
Europe	64	58	30	100	18	72	16	78	69	99	142	89
Middle East	554	53	10	100	72	68	20	100	10	100	5	99
Organisation for Economic Co-operation and Development Pacific	23	77	130	100	9	90	15	74	116	97	198	99
Other Developing Asian Countries	38	51	5	100	14	55	12	78	34	84	142	92
USA	99	52	650	100	19	75	20	50	556	99	317	95
Global	1417	54	2445	100	257	69	247	82	2462	96	1438	95

'Conven' and 'Unconven' mean conventional and unconventional resources, respectively.

Fossil fuels should be given due credit for providing countries with the opportunity to shift into spheres of unparalleled economical, social and political influence (Council 2007).

Petroleum is absolutely crucial to the transportation sector, while coal is to be recognized as the major source of the world's electricity production. Though, it soon became evident that the rate at which the standard of living was constantly being upgraded meant that the demand for energy just kept on soaring (Raupach et al. 2007). Fossil fuels have very effectively continued to meet this demand and have allowed us as a species to advance and develop more rapidly than what was ever thought possible (Nel and Cooper 2009).

The vigour of the global economy and energy security is rather questionable at best. Almost all commercial processes, products or services rendered are directly or indirectly dependant on conventional energy (Bilgen 2014). In reality this means that the maintenance and development of the global economy is founded on a commodity which is being rapidly exhausted, without the hope of its own replenishment. A major drawback, which strings even more tightly the cord which holds everything together, is that fossil fuels are very sparsely distributed throughout the world. A significant portion of this energy then needs to be set aside just for the fruitless task of transporting and distributing the remaining energy throughout the world (McGlade and Ekins 2015).

2.2 Energy: current consumption and predictions of future requirements

Improving the efficacy of alternative energy production has become of paramount importance if society is not to regress to the pre-fossil fuel era as a result of dwindling energy supplies. Since 1990, global energy consumption has been amplified by two factors. Firstly, developing countries have been gradually increasing their consumption of energy in order to sustain their current trends of development. Secondly, development requires and also allows for greater amounts of energy to be consumed. The combined effect of both these factors resulted in the tripling of energy consumption per capita from 1990 to 2014. Unfortunately, estimates predict that global energy consumption will carry on increasing and rise by a further 56 % by 2040 (Bilgen 2014).

In 2008 more than 85 million barrels of oil was consumed globally everyday, which equates to 31.2 billion barrels over that year. The International Energy Agency (IEA) estimates that by 2030 the annual consumption of oil will have reached 42.5 billion barrels per year (Owen et al. 2010). In 2010 global energy production related CO_2 emissions stood at 31.2 billion metric tons for the year. Predictions estimate that by 2020 and 2040 this figure will increase to 36.4 and a staggering 45.5 billion metric tons for the year, respectively (Bilgen 2014). At the current rate, conventional oil fields will only be able to supply 50% of the global oil demand by 2020 (Bilgen 2014). In order to meet the heavy demand for oil, production had to move to unconventional sources (Salameh 2003) such as tight and recoverable oil. These oils are found within reservoirs which do not allow for economical flow rates of oil to the wellbore after drilling (Chengzao et al. 2012). This is thus a more expensive product to produce and part of the reason why oil prices are on the increase (Murray and Hansen 2013).

2.3 The gravity of the fossil fuel dilemma

The limited amount of conventional energy which can be utilized while aiming to remain within an "acceptable" range of global warming is a very obvious setback. In fact, misreporting of ample global reserves has been employed to falsify data entering the public domain in order to create incentive to ultimately gain market share, as was admitted by the

IEA (Owen et al. 2010). This is the degree of desperation which diminishing reserves have been dealt with. This desperation, to a large extent, finds its origins in the drying up of the world's life-giving oil fields. The loss in productivity of these fields is illustrated in Figure 2.1, where the drop-off in supply of fields currently in production can clearly be seen. Global oil supply is produced from 70 000 different oil fields and a mere 110 of these fields are responsible for 50% of the world's conventional oil supply. Of the 507 fields considered as "giants", more than half have been experiencing a decline in production since 2007 (Owen et al. 2010). However, the true dilemma lies in how destructive the production and use of fossil fuels truly are with respect to the environment and even to life itself. There is not a single environmental advantage to producing and consuming fossil fuels, in fact, quite the contrary is true. Land degradation, soil, air and water pollution, health hazards, habitat destruction, climate change and global warming are all notions strongly linked to the use of fossil fuels (McLaughlin 1985, Mukhopadhyay and Forssell 2005, Khoo and Tan 2006).

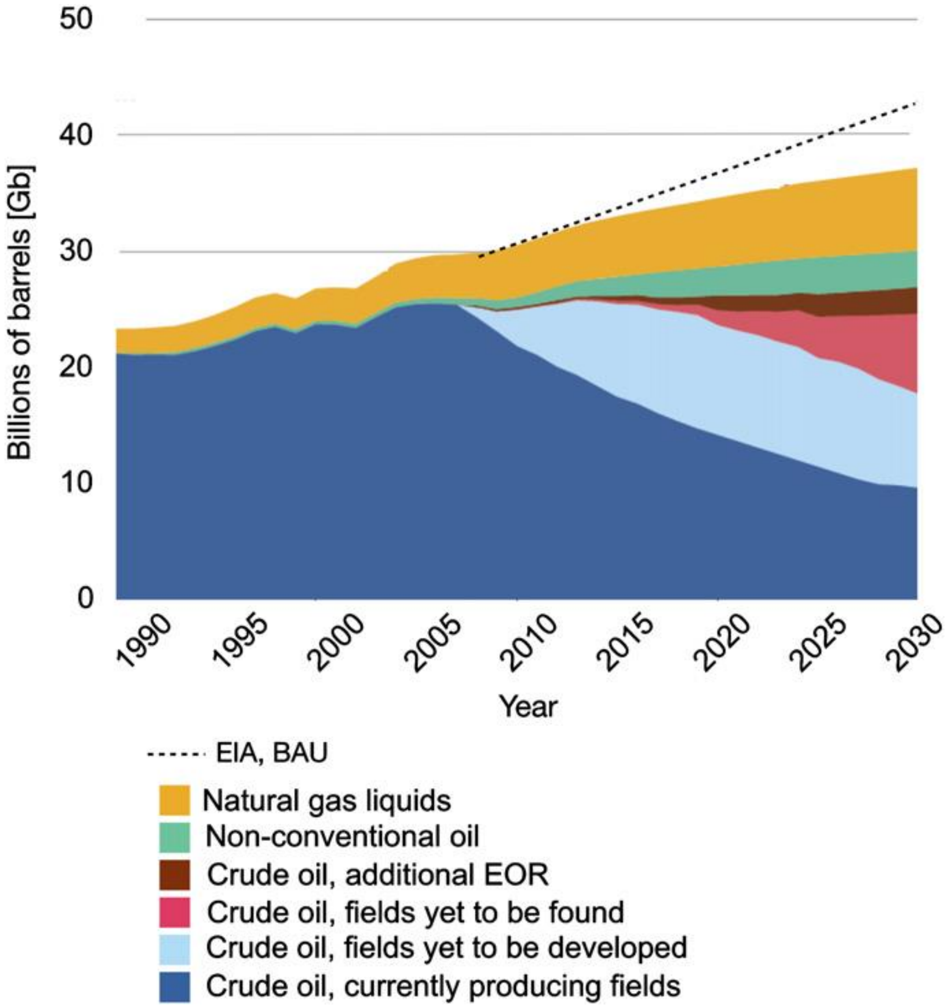


Figure 2.1: Projected world liquid fuels global demand and supply (Owen et al. 2010).

Two notorious compounds of fossil fuel combustion are nitrogen oxides (NO_x) and sulphur oxides (SO_x), which are emitted in vast amounts, especially by coal-based electricity production plants (Hoel and Kverndokk 1996, Reible et al. 2016). These pollutants are problematic in that they oxidise in the atmosphere, turn to acid and then fall to the surface of the earth as acid rain. They are also major components of the green house gas (GHG) mixture and contribute very negatively to global warming (Bilgen 2014). These two compounds undoubtedly represent but only a fraction of fossil fuel pollutants and associated effects in their entirety.

2.4 Substituting fossil fuels

The urgency with which global energy dependence will shift towards renewable energy is dependent on many factors. These include oil price hikes, price volatility, levels of emissions, foreign energy dependence, environmental factors and environmental consequences (Bilgen 2014). In a nutshell, this relates to energy security and climate change, which embody two of the key concerns that need to be dealt with currently and into the foreseeable future. Sustainability of healthy economies, countries and ecosystems the world over, will depend on secure and steady supplies of clean, renewable energy. Maintaining such sustainability necessitates of the human race to encourage many changes and to do so in a relatively short period of time. However, clinging to the familiar, coupled with intense pressure and desperation of each country to safeguard its own interests will more than likely lead to regional and global conflict in order to attain and secure remaining conventional energy reserves. Therefore, the faster the threat of limited and declining energy is neutralized, the faster the volatility of the situation can perhaps be subdued.

In order to shift from conventional to renewable energy as smoothly as possible, accurate estimations need to be made on how much fossil fuel energy can be budgeted for and at what rate it will most likely be consumed (Chow et al. 2003). Due to different levels of development as well as differing regional supply capacity, each country or region has a different level of supply and demand. It is very important to have a good understanding of both demand and supply of each region in order to assess its level of dependence on foreign fuel as well as its ability to sustain itself with its remaining energy resources (Bilgen 2014). With this information at hand it is then possible to assess which forms of alternative energy would be most feasible, practical and profitable for a region. However, all influential factors, including climate, landscape, indigenous flora, infrastructure and types of energy most utilized (e.g. solid fuels, gas etc.) should all be considered and regarded before the best suited forms of alternative energy could be implemented.

2.5 Alternative energy forms and sources

Many possibilities for clean sustainable alternative energy exist, such as wind, hydro, geothermal, nuclear and solar energy, and all have been proven to have potential if implemented correctly. In the future each of these will most likely play a vital role in reaching the enormous global energy demand. It cannot be denied however, that each of these comes with its own drawbacks and shortfalls, such as intermittency and high cost of establishment and maintenance (Banos et al. 2011), but the drawback which unites them all is that these systems can only generate electrical energy. Regrettably, these alternatives will thus play a futile role in quenching the world’s thirst for oil.

Luckily, bioenergy will be able to offer some relief to the global oil demand, since all forms of energy used on a daily basis, or at least alternatives to them, can be fabricated through processes of bioenergy production. Emphasis should, however, be drawn to the fact that the best production design for each region needs to be implemented, instead of simply defaulting to a single design and applying it to all scenarios. This approach might assist in environmental and energy objectives actually being met (Schubert 2006).

2.5.1 Bioenergy: problems and solutions

According to Bilgen (2014), renewable forms of energy are efficient in energy production, free of health hazards, pollution and environmental degradation. Though this may be true under certain circumstances it is most definitely not always the case. Ignorance can easily be used to mislead the uninformed, and the field of bioenergy is by no way exempt from this trickery. In the case of bioenergy both extremes have taken root, where biofuels are unfairly dismissed and also where they are unjustly glorified.

Too often publications and articles have outright crucified biofuels and labelled them as “bad” and unfeasible, all based on a skewed point of view. For instance, America jumped onto the biofuel bandwagon, mandating biofuel blends in order to reduce greenhouse gas emissions and move towards cleaner energy (Stankus 2014). This was a noble campaign with excellent motives, but unfortunately yielded poor to perhaps even negative results. Ethanol, the alternative to petrol, produced by maize in this case, has only about two-thirds the energy density of petrol (De Oliveira et al. 2005). That effectively means that less mileage is attained per tank of ethanol as compared to petroleum. This would result in filling up more frequently, which is slightly inconvenient but generally not a significant problem. The real issue is that maize is a very poor first-generation feedstock (biomass source used to produce bioenergy), and that the process of producing maize-based ethanol uses virtually as much energy as it produces (de Vries et al. 2010). From this point of view biofuels are indeed “bad” because these crops are inefficiently utilising resources and land which could otherwise have been used to produce food. It is on this point that many people get stuck and lose faith; however, this isn’t the end of the line for biofuels. So many, more efficient and environmentally friendly feedstocks other than maize are available. The substitution towards these feedstocks would drastically improve feasibility and correct the misperceptions surrounding biofuels.

Clearly, bioenergy itself is not without its own disadvantages and hurdles, however, many of these could be solved fairly easily. For instance, the fight over using food crops for food or fuel production can be largely resolved by simply shifting the bulk of bioenergy production from food crops (first generation) to non-food crops (second and third generation) instead.

Naturally, there is also the conflict which presides when using arable land to grow energy crops to keep the lights burning instead of food crops to feed a nation’s people, and in certain cases the ethics around this issue should most definitely be given priority. However, the demand for land often needs not be a problem because ample marginal, unused and degraded land exists which can be utilized for energy production (Liu et al. 2011). These poor quality soils could perhaps even be improved or rehabilitated by gaining organic matter and being buffered against erosion (Carter 2002). Also, second generation crops produce much greater quantities of biomass than the soluble carbohydrates produced by most first generation crops, and therefore if good quality soils were to be used, theoretically, much less land would be required for the same energy output (Sanderson and Adler 2008). The portion of arable land set aside for energy production thus truly need not encroach heavily on land essential for food production.

Furthermore, it has been found in the past that when there was surplus of certain types of fresh produce, such as potatoes, then culling was implemented and justified against the cost of processing (Treadway 1947). Economically this probably makes sense, but the ethicality thereof is uncomforting. If a percentage of good quality land is used to grow bioenergy crops, then the ethical issue of surplus food and the culling thereof will to a large degree possibly solve itself, as well as the drop in food profit margins owing to surplus.

In many countries water scarcity is a pressing problem. Unfortunately, whether mining for conventional reserves or producing bioenergy through agriculture, water is going to be required. The difference is that mining generally tends to pollute water sources, whereas bioenergy production has the potential to clean water and even reduce water losses (Bliss et al. 2009). An example is algal turf scrubbing, a procedure which uses algae to purify water of nutrients and even certain pollutants, all while producing vast amounts of bioenergy feedstock (Adey et al. 2011). Dense plant populations can also drastically reduce rain water runoff and facilitate better water infiltration into the soil and so aid in refilling underground water banks (Pinners 2014). Biofuels, as the name implies, are biological and therefore also biodegradable, and so if spilled, will not threaten water systems for enduring periods of time (Zhang et al. 1998). A possible advantage which can be exploited is that many plant species indigenous to an area are often very hardy and water use efficient (Boyer 1982). Different species can therefore perhaps be successfully cultivated on dryland, drastically lessening the pressure on a region’s water reserves.

There is some concern and uncertainty about whether bioethanol can be used directly in combustion engines or not, and if so how they may affect the longevity of the motor. The answer is quite simple. All petrol motors can run, unaltered, on bioethanol and petroleum blends of up to 20 % bioethanol (Demirbas 2008). The reason for this low concentration is that bioethanol contains molecules which reduce the elasticity of rubbers and plastics. Higher percentage blends will / can thus result in rubber components such as fuel lines becoming brittle and failing (Haseeb et al. 2010). All that is required to completely resolve this problem is to replace these parts with components designed to resist degradation. A practical example of overcoming this hurdle is seen in Brazil where 70% of new cars sold are flex-fuel vehicles (Wilkinson and Herrera 2010); meaning that they are able to run on either petroleum or ethanol or any blend ratio of both.

A major hurdle faced by bioenergy production is that by weight, biomass has a fraction of the energy density of fossil fuels (Erol et al. 2010). This challenges production feasibility because biomass generally needs to be harvested and transported over long distances to where it will be used or processed. This energy expenditure needs to be justified by net energy production and overall production costs. The fact that raw biomass has such a low energy density compared to fossil fuels means that there is a narrower margin within which bioenergy production will remain feasible. Fortunately, there is a process which may offer a solution to this problem – pyrolysis.

Pyrolysis is the exposure of biomass, for only several seconds, to high temperatures of between 400 and 700 ^{o}C under anaerobic conditions. Different forms of energy dense products can be produced, such as biogas, bio-oil and biochar (Shafizadeh 1982). Fresh biomass converted to bio-oil can become up to seven times as energy dense as in its raw state and therefore, per unit energy, the weight and volume of biomass is drastically reduced once converted (Badger and Fransham 2006). It can be reasoned that if pyrolysis of biomass could be done locally before transportation, the margin within which profitability remains feasible would increase substantially. If this was coupled with refinery or processing to produce butanol, then feasibility might even rise to a whole new level. Butanol is an alcohol, similar to ethanol, with the exception of two major characteristics. This biofuel can be used in any concentration without any engine alterations, and it has an energy density virtually equal to that of petroleum (Dürre 2007). Such a course of action could put renewable energy on par with fossil fuels, less the environmental destruction.

Biochar is an extremely stable form of activated carbon and can persist in the soil for thousands of years, far longer than other known forms of organic carbon. Biochar has a very high capacity for mineral adsorption, and because these minerals are in an exchangeable state they remain plant available. This material has the potential to improve soil nutrition, soil properties and water holding capacity, reduce fertilizer requirements and decrease nutrient leaching into groundwater (Lehmann 2007). A very high carbon recovery of 50% is generally achieved when producing biochar, due to the increase in carbon concentration when converting biomass into biochar. This means that amending soils with biochar is not only highly beneficial to the soil itself, but it also offers a means of extracting carbon from the atmosphere and effectively storing it in the ground. This application could make for a carbon negative bioenergy production procedure (Lehmann 2007). Biochar is also able to scrub NO_x, SO_x and CO_2 from flue gas (the gas mixture produced by industrial plants or power stations due to the combustion of fuels such as coal) by precipitating these compounds onto its surface. Scrubbing with biochar could thus be used in conjunction with coal firing in order to scrub flue gas before it is released into the atmosphere (Brown 2009).

A reality that will most likely arise when producing bioenergy is that the maximum amount of biomass available will be removed from an area, whether it is specifically grown biomass feedstock or designated crop residues (Crocker and Andrews 2010). This will probably be necessary in order to make production as profitable as possible and to meet global demand. The side-effect thereof is that soil productivity will decline due to degradation and constant removal of organic matter (Carter 2002). Certain by-products of bioenergy production offer many beneficial attributes and could be used to amend both arable and poor quality soils. For instance, the process of making biogas requires biomass to be fermented in a bio-digester. Once the gas is extracted, a digestate high in minerals, nutrients and organic matter remains, which can be used as a natural fertilizer (Akiyama and Tsuruta 2002, Tambone et al. 2010). These two by-products, biogas digestate and biochar, have the potential to increase crop yields, while reducing pollution and extracting GHGs from the atmosphere (Chan et al. 2008, Ippolito et al. 2012).

As previously stated, an enormous drawback with regards to fossil fuels is how scarcely major sources are distributed throughout the world. The advantage offered by bioenergy is that of native potential and hardiness. Every country or region has flora species which are indigenous to the area and therefore well adapted to handle the climatic and environmental elements posed by that region. These species could be harnessed and exploited to develop energy systems best suited to each area and thereby offer a great degree of liberation from the dependence on foreign energy (Davidson et al. 2011).

Many researchers believe that a feedstock needs to be distributed over a wide geographical range for it to have good potential for commercial energy production. This is definitely advantageous in the sense that localized cellulosic refineries could be fine-tuned to a single feedstock in order to attain maximum productivity. However, the underlying problem is that countrywide and perhaps even semi-continental monoculture could be established. Yet, the renewable fuel standard under the EISA (energy independence and security act) of 2007 mandates that over 60 billion litres of cellulosic ethanol be produced in the USA by 2022, with switchgrass as the primary feedstock (Rogers et al. 2014). One does not have to look very far to find good examples of bad outcomes as a result of monoculture. Pests, diseases, narrowing of the gene pool and climate change are all time-bombs waiting to cause an epidemic, and unfortunately the greatest degree of instability arises when monoculture comes into play (Andow 1983, Cannell 1999). Besides, monoculture may not be as advantageous as generally thought. Researchers have found that the interaction between different grass species in natural prairies resulted in almost double the biomass yields as compared to when switchgrass was grown as a monoculture. The various interactions allow for every possible niche to be filled and to more effectively fend off climatic threats such as drought (Schubert 2006).

2.5.2 Bioenergy – a maturing field

First generation biofuels, as already stated, are generally frowned upon. They have also been widely speculated to be a major factor influencing the rise in food prices. Allegedly, the maize and soya which has been set aside for bioenergy production has left sectors such as meat and fish production with an unsatisfied demand for maize- and soya-based animal feeds, resulting in food price hikes. Additional concerns such as increased rather than reduced GHG emissions, high water and fertilizer demands, and loss in biodiversity due to extensive monoculture are factors which could be faced by poor management decisions (Stankus 2014). One will almost always find that first generation crops are subject to these shortfalls, largely due to the fact that the components of these crops used to produce energy are only a fraction of the entire plant.

Second and third generation crops are both classified as non edible crops, with third generation referring specifically to algae. Cellulosic ethanol is manufactured from the cellulose, hemicellulose and lignin fractions of biomass produced from second and third generation crops. These three components are collectively referred to as lignocellulose and make up the bulk of all plant matter and therefore is also the most abundant form of biological matter the world over (Kuhad and Singh 1993). Unfortunately, lignocellulose is much more challenging to extract compared to soluble carbohydrates and sugars and therefore more intensive and expensive sugar-liberating processes, known as saccharification, are required (Mosier et al. 2005). However, cellulosic ethanol production generates 4 – 10 times the amount of energy that it consumes, compared to the humble 1.3 energy ratio of first generation maize ethanol. Also, the full life cycle of maize ethanol only reduces GHG emissions by a shy 13%, compared to that of cellulosic ethanol with a reduction of 85% (Schubert 2006). Meanwhile, as technology is being improved and conversion processes made more efficient and less costly, identification of feasible bioenergy crops is to be made a priority.

Ever increasing concentrations of second generation biofuel blends as required by mandates are currently heavily dependent on subsidies to cover their production costs (Lapan and Moschini 2012). At present, the lack in feasibility of these biofuels may result in their increased production being frowned upon. However, the reality of a fossil fuel budget cannot be ignored and therefore the current financial investment in the improvement of bioenergy procedures and technologies will most certainly pay off in the future. Further assurance and security in the bioenergy venture is supported by Nigam and Singh (2011), who stated that on a short-term basis especially, biofuels are the most promising alternative to fossil fuels because of their state of market maturity as compared to other alternatives.

Also, according to Stankus (2014), the greatest portion of biomass used to produce second generation bioenergy is in actual fact residues of existing food crops, as can be seen in Figure 2.2. This is a constant and ever growing source of biomass which requires the same saccharification procedures as second generation crops if they are to be used to produce liquid fuels. The improvement of second generation bioenergy technology will thus add significantly to the overall energy productivity of food production as well, and not merely just to direct second generation energy production. An interesting fact is that maize stover and animal manure are considered to be some of the greatest disposal challenges faced by America (Stankus 2014). Improvement in second generation technology could provide an effective solution to this problem, while doing a part to alleviate the global energy crisis.

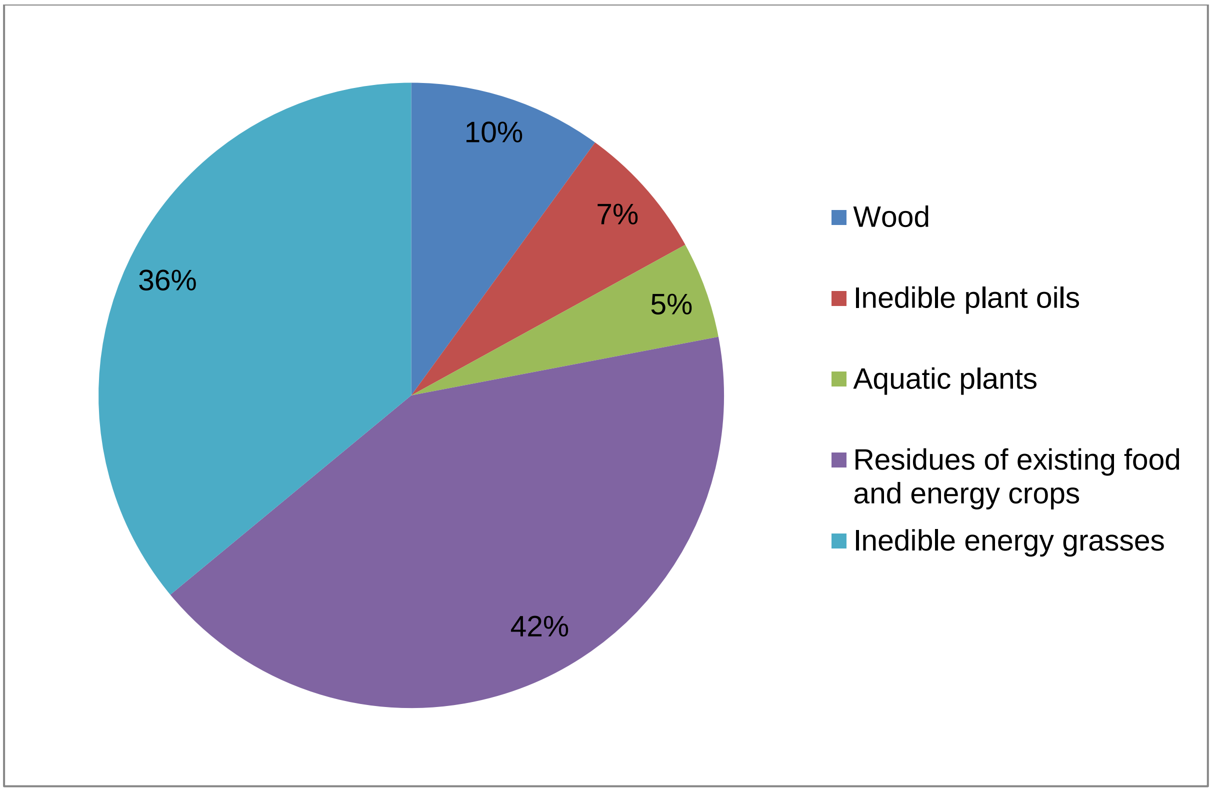


Figure 2.2: Plant sources of second generation biofuels by general category. Reference made to 263 plant-originated renewable energy feedstocks in titles and keywords in 300 articles from the Web of Science (Stankus 2014).

It is strongly suspected that trends of declining oil prices will result in a decline in interest of renewable energy development. This is a mind-set which cannot be allowed to linger. Currently arguably the greatest incentive for bioenergy production is the mandating of ever increasing biofuel blends to petroleum fuels (Sorda et al. 2010). Unfortunately, the biggest driver of bioenergy production is as of yet not the potential for incredible efficiency and profitability of this venture. There is, however, a big upside for the future of bioenergy production and that is that in almost every single area of this field there is still room for improvement (Yuan et al. 2008, Senger 2010, Dererie et al. 2011). This should be a beacon of hope because many forms of bioenergy production have already proven themselves as more than capable of helping to cut back on the world's fossil fuel dependence. Any improvements will thus continue to aid in the strengthening of bioenergy potential and productivity going into the future. Such improvements may also be in much closer reach than anticipated, because plants have not historically been bred for high biomass production. For instance, according to Sticklen (2006) a single generation of conventional breeding and selection of shrub willow, specifically aimed at increased biomass production resulted in a 40% greater biomass yield.

Furthermore, a fair point to keep in mind is that when conventional oil production was in its infancy, it was a lot more expensive than what it is currently (Schubert 2006). In fact, a massive 70% of the cost of cellulosic ethanol production lies in the conversion processes and only 30% in the biomass production itself. This cost distribution is exactly the opposite of what is experienced by today's oil refineries (Schubert 2006). It is clear that there is ample room for improvement in conversion technology and therefore just as much room for increased profitability in the bioenergy field.

2.6 Righting the wrongs of energy consumption and production

With all that has been said, estimated and speculated, the first order of business still remains. Reducing wasteful energy consumption is probably the simplest manner of immediately alleviating fossil fuel demand and environmental degradation. This can to a great degree be achieved by educating the masses and making them aware of where and how they can reduce energy consumption in their daily lives. A disturbing fact is that a staggering 90% of the world's fossil fuels are consumed by a mere 10% of the world's population (Bilgen 2014). This surely infers an enormous amount of wastage or unnecessary consumption throughout the domestic and industrial spheres of society (Banos et al. 2011). Without addressing this aspect of energy consumption, rapid and lasting changes to global energy use efficiency will never be fully achieved.

In terms of environmental wellbeing, humans need to take responsibility for their actions and do their part in attempting to rectify the wrongs they have done to the planet. Vast amounts of natural forests are being uprooted in order to plant bioenergy crops such as sugarcane and palm oil (Schubert 2006). These forests, natural and planted, undoubtedly play a major role in mitigating CO_2 released into the atmosphere. Destroying nature with the hope of aiding its wellbeing is a rather blind approach. Therefore, natural forests need to be protected, forests that were cut back need to be replanted and new forest areas need to be designated and established (Bilgen 2014) so that humanity might slowly increase the earth's carbon mitigating capacity, for its sake as well as our own.

2.7 Important attributes of a bioenergy crop

According to Henry (2010) and Stankus (2014), an ideal bioenergy crop should produce high biomass and energy yields, be easily planted and established, preferably be perennial, have no or little invasive traits, proficiently grow on marginal land, require little or no supplementary water or fertilization, effectively anchor the soil, allow for multiple harvests throughout the year, and be able to be stored in-field after harvest. Furthermore, they should show production feasibility when produced on large-scale, produce valuable co-products, have potential for genetic modification, have high nutrient partitioning to roots and non-harvested components and effectively extract carbon from the atmosphere. Bioenergy crops must not require an extensive skill set to be managed, their resource requirements and pre-treatment costs must be justifiable, environmental and ecological effects must be as advantageous and non-threatening as possible, feedstock availability must allow for year round energy production, and the fuels produced must easily be incorporated into the energy network already in use (Stankus 2014). The influence of these attributes can easily be underestimated, however, as explained below, their effects and outcomes can be far reaching.

Studies have determined that within the top 3 m of soil, many perennial grasses deposit carbon at an average rate of 1.1 ton ha^{-1} yr^{-1}. A further 3 to 4 tons ha^{-1} yr^{-1} was found to be added when turnover of roots and microorganisms in the soil was incorporated into the calculation (McLaughlin and Walsh 1998). The addition of this soil organic matter (SOM) improves soil properties and structure, limits soil erosion, runoff and leaching of nutrients, and also positively affects soil density, water holding capacity, nutrient availability and aeration (Carter 2002).

During heavy rain spells soil erosion for row crops such as maize have been documented to be in excess of 200 times the amount from perennial grass fields. In America, tens of billions of dollars of fertilizers are lost annually as a result of soil erosion. Therefore, enormous economic and environmental benefits stand to be gained or saved by the replacement of annual row crops with perennials such as switchgrass (McLaughlin and Walsh 1998).

2.8 Currently used feedstocks

An extremely wide range of different feedstocks have been identified and used to produce bioenergy. These species span and overlap a geographical range which covers an enormous portion of all terrestrial surface area (Offermann et al. 2011). However, after further analysis many of them are rendered futile because they do not meet the important aspects necessary for proficient bioenergy production. Important attributes of a bioenergy feedstock have already been mentioned and it is clear that the extent of these traits is extremely vast. For this reason, research focus of the present study was placed on only a selected few parameters, namely biomass yield, water use (WU), water use efficiency (WUE), CV and leaf area index (LAI).

Biomass production and the calorific value (the amount of energy produced when a set quantity of dry matter is combusted in a closed system such as an oxygen bomb calorimeter), are aspects of paramount importance with respect to bioenergy production because they relate to the annual total harvestable energy from each hectare of land. This, before all other abilities, qualities or shortfalls carries the most weight with regards to proficient bioenergy production. However, harvestable energy is only a large part of the bigger picture, and not the sole factor to be taken into consideration. For instance, the application of water often comes at a significant cost. In addition to these costs, it is estimated that almost 70% of global water withdrawals are consumed by the agricultural sector, and this percentage is expected to rise with increasing bioenergy crop production (Erickson et al. 2012). Therefore, specific selection of bioenergy crops is of particular importance to ensure high WUE in conjunction with high overall energy production. A good reference point to work from when considering the WUE of prospective bioenergy crops is the review compiled by Stanhill (1986), which reported that over a range of 14 different C4 species the average WUE was found to be 31 kg DM ha^{-1} mm^{-1}.

Even though light energy is freely available it should not be taken for granted or used wastefully. According to Guenni et al. (2005) most grass species intercept 80 - 85% of PAR (photosynthetically active radiation) when the LAI is between 4 - 5 m^2 m^{-2}. This is an important feature because a low LAI generally leads to low fractional solar radiation interception and therefore potential energy wasted and inefficient land and water use. The efficiency of radiation interception will also be affected by both the vertical distribution of leaf area throughout the canopy, as well as the structural design of leaves themselves, otherwise collectively referred to as canopy architecture. An example of desirable canopy architecture is that found in Napier (*Pennisetum purpureum*), where the erect growth habit of the leaves allows for better light penetration into the canopy. This feature is credited as an important reason for Napier's high biomass yield, and is a highly advantageous trait in energy grasses (Kubota et al. 1994).

Several of the more common or well-known feedstocks, along with some pros and cons for each are mentioned below. Unfortunately, for several species information is limited or completely lacking with regards to the parameters in question. However, even though fragments of information are void, a single recurring piece of information is to be noted, and that is that many of the species which exhibit strong traits of a proficient bioenergy feedstock are part of the *Poaceae* family.

2.8.1 First generation feedstocks

2.8.1.1 Sugarcane *(Saccharum officinarum)*

In 2006 Brazil had already converted 30% of their energy matrix to biomass based energy, with sugarcane being responsible for 15% of the country's total energy supply (Wilkinson and Herrera 2010). At that time a relatively small area of land was dedicated to sugarcane cultivation for ethanol production as compared to land used for food production. Yet, Brazil had already managed to do away with a large portion of their fossil fuel dependence. According to Richardson (2010), South Africa shows very similar potential to be able to substitute a significant portion of fossil fuels with bioenergy, while keeping the land-use balance between food and fuel always tipped towards food production.

Brazil's commitment to ethanol production resulted in improvements in all related fields of sugarcane and ethanol production, including agricultural practices, seed, equipment, processing technology, and machinery. These constant improvements have led to an annual increase of 4% in overall productivity since the 1970's to the extent that Brazilian ethanol is now competitive with petroleum, even with oil at a low price of $30 per barrel, which makes ethanol so productive that subsidies are no longer required (Wilkinson and Herrera 2010). All of the above are indicators which support the idea that bioenergy investment will be well worth while in the long run.

Sugarcane bagasse is often burnt on many sugarcane farms the world over to supply steam power to machinery and to concentrate pressed cane juice. This adds to the energy efficiency of the farm, but research shows that low-technology, small-scale combustion operations emit more GHGs than what bio-refineries would when producing cellulosic ethanol from the bagasse. However, burning of bagasse is already a step in the right direction because it releases less GHGs as compared to burning lignite, which is the main fuel source of Asia and most of Africa (Stankus 2014).

In Brazil sugarcane has been found to occupy a root zone of 4.5 m after only one year of establishment (Knoll et al. 2012), which is extremely advantageous during water-stressed periods. According to Botha (2009), record sugarcane dry matter yields have been recorded at 80 - 85 tons DM ha^{-1} yr^{-1}, with average yields expected to be around 39 tons ha^{-1} yr^{-1}. Olivier et al. (2015), however, reported yields of 70 tons DM ha^{-1} yr^{-1}, much greater than the expected averages reported by Botha (2009). It is likely that the differences in average yields reported by Botha (2009) and Olivier et al. (2015) are due to yields attained under dryland and irrigated conditions, respectively. The calorific values of bagasse and water soluble carbohydrates such as sucrose were 17.4 and 14.8 MJ kg^{-1}, respectively. The annual energy yield of sugarcane, averaging at 679 GJ ha^{-1} and peaking at 1479 GJ ha^{-1} is significantly greater than that put forward for switchgrass.

A relatively high LAI, averaging between 6 - 7 m^2 m^{-2}, should also result in high radiation interception. A lot of sugarcane's biomass is situated in the stems because its LAI is lower than that of switchgrass for instance, but its dry matter yield is exceptionally greater (Teruel et al. 1997). Thus, from a genetic point of view it may prove highly beneficial to specifically breed energy grasses with the aim of increasing the stem to leaf ratio of biomass.

A local study which compared the WUE of sugarcane with that of popular bioenergy crops found that conventional sugarcane, with a dry matter WUE of 60 kg DM ha^{-1} mm^{-1}, was surpassed only by sorghum (Olivier et al. 2015). The total water use of sugarcane in this trial varied between 975 – 1100 mm for the water stressed treatment. The harsher growth conditions created by both lower rainfall and higher vapour pressure deficits (VPD) of many marginal areas might however not deliver the same verdict with respect to WUE (Seneweera et al. 1998). Thus, further trial work will first need to be conducted before sugarcane can be considered for these regions.

2.8.2 Second and third generation feedstocks

2.8.2.1 Switchgrass *(Panicum virgatum L.)*

Switchgrass is seen in a very positive light with regards to sustainable renewable energy production, and this is due to its wide range of positive attributes. Such attributes are of paramount importance when deciding which feedstocks to produce on large enough scale to address regional and global energy production.

In the natural prairie systems of America switchgrass has an impressive average root length of 2.6 to 3.7 m. Its perennial growth habit aids in the development of this enormous root system and ultimately to its efficient nutrient absorption and low supplementary nutrient requirements. The expansive root system is also a massive source of soil organic matter (SOM), nutrient reserve for stressful years and a very effective carbon sink (Schubert 2006).

Herbicides are usually applied to switchgrass only during the establishment year and then not again for the entire length of at least a decade's life cycle. The financial and environmental advantages thereof are monumental (Schubert 2006).

According to research done by McLaughlin and Walsh (1998) and Schmer et al. (2008), ethanol production from switchgrass provides a 343 - 540% net energy gain, whereas maize ethanol only provides a 21 - 33% net energy gain. The reason for the tremendous energy gains with switchgrass as compared to maize is the lower energy requirements at each step from production all the way through to conversion. The procedure of converting switchgrass, to ethanol, when combined with underground carbon storage in roots, resulted in 30 times less CO_2 being emitted, compared to when maize was used as a feedstock.

Switchgrass produced average dry matter yields of between 5.2 - 11.1 tons ha^{-1} yr^{-1} (Schmer et al. 2008), with a calorific value of 18 - 19 MJ kg^{-1} (Sadaka et al. 2014). These values may result in annual harvestable energy per hectare ranging between 93.6 - 210.9 GJ ha^{-1}, which is significantly lower than that of many other energy species. However, the fact that switchgrass is still considered a prime bioenergy species supports the notion that energy production alone does not determine the proficiency of a species.

The dry matter water use efficiency of switchgrass was recorded at 25 kg ha^{-1} mm^{-1} for summer-growth, according to Stout (1992). McLaughlin and Kszos (2005) did, however, attain much higher WUE values of 43 - 85 kg DM ha^{-1} mm^{-1}, which seem to be more in line with literature. The species' fairly high LAI of 6.1 - 8 m^2 m^{-2} is also highly advantageous and should be sufficient to intercept virtually all PAR (Madakadze et al. 1998).

2.8.2.2 Energy cane *(Saccharum hyb.)*

Energy cane varieties are produced by crossing cultivars of commercially produced sugarcane with related wild species. These varieties are specifically cultivated for bioenergy production and are generally more cold tolerant, resource use efficient and have greater fibre content than conventional sugarcane. These cultivars could thus effectively fill niches such as areas with marginal land and poor quality soils (Shields and Boopathy 2011).

A moderate annual dry matter harvest of 20 ton ha^{-1} under dryland conditions with no fertilizer supplementation was recorded by Knoll et al. (2012), but much greater yields of 70 ton ha^{-1} yr^{-1} have been achieved under more favourable conditions (Olivier et al. 2015). According to Waclawovsky et al. (2010), a theoretical maximum annual yield of 177 ton ha^{-1} yr^{-1} is achievable. Additionally, water and nitrogen application can be specifically managed, as well as the harvest period brought down to six months instead of 12, in order to increase biomass yield instead of sucrose production (Olivier et al. 2015).

Energy cane, with a seasonal water use of 1629 mm (Olivier et al. 2015), used even more water than conventional sugarcane (Dufey 2008), however this statement should be qualified, because overall energy yield and production costs should also be brought into the equation. The higher fibre content and the apparent high resource use efficiency may lead to cheaper energy than can be delivered by conventional sugarcane, even with a lower WUE of 45.4 kg DM ha^{-1} mm^{-1} (Olivier et al. 2015).

The fair LAI of 4 - 5 m^2 m^{-2} (Na et al. 2015) might present an area which can be improved upon by simple practices of conventional selection and breeding. Such improvements might lead to energy cane cultivars with improved fractional interception of PAR and so, perhaps even greater biomass yields.

The calorific value of energy cane seems to form part of the fragments of information lacking in this field, and therefore make for difficulty in estimating total annual energy production of this species. The assumption could however be made that the CV of energy cane might be greater than that of conventional sugarcane as a result of an increased fibre content. However, for the purpose of merely estimating an energy yield, if the same CV of sugarcane is assumed for energy cane, then an energy yield of 348 - 1218 GJ ha^{-1} yr^{-1}, and a theoretical yield of up to 3079.8 GJ ha^{-1} yr^{-1} could be expected.

2.8.2.3 Bamboo *(Phyllostachys beterocycla)*

Bamboo has an extremely wide geographical distribution and is used in many different applications throughout the world (Hunter 2003). Its versatility significantly adds to its potential and production feasibility because it is valued in many other sectors of the bamboo trade (Marsh and Smith 2007).

The resilience of bamboo fibre to degradation requires that even more harsh pre-treatments than for most other cellulosic feedstocks be applied. However, it has recently been found that the addition of bamboo charcoal to bamboo slurries resulted in the absorption of naturally occurring compounds in bamboo which are responsible for its resistance to fermentation (Stankus 2014). Environmentally, bamboo used as a feedstock for biofuel production is very gentle because it results in very low levels of noxious compounds being emitted into the atmosphere (Stankus 2014).

Average bamboo dry matter yields seem to converge around 49.5 tons ha^{-1} yr^{-1} (Hong et al. 2011). The reported CV of 17.6 MJ kg^{-1} (Sridhar et al. 2007) would thus result in an enrgy yield of 871.2 GJ ha^{-1} yr^{-1}. Patil et al. (1988) attained a fair LAI of 4.77 m^2 m^{-2}, which might, similarly to energy cane, be an aspect that is open to improvement through breeding. However, according to Scurlock et al. (2000), amidst these desirable characteristics lie several challenges or shortfalls, which most certainly forestall the expansion of bamboo utilization in the bioenergy field. Very limited literature is available with respect to bamboo production and even that which is available does not seem to suggest that greater biomass yield potential is to be gained by bamboo production as compared to many other bioenergy feedstocks. It seems that the “average” biomass gains posed by bamboo production do not offset the difficulty of selective breeding and lack of knowledge with respect to propagation, stand establishment, management and mechanized harvesting of this species (Scurlock et al. 2000).

2.8.2.4 Tree species

Soft and hardwood trees are also amongst the energy crops under discussion and can be used to produce ethanol, biodiesel, methane, wood pellets etc. Willow, Poplar, Sweetgum, Sycamore, Jatropha and Eucalyptus are popular tree species which are either being tested for bioenergy potential or are currently already being used as feedstock (Kole et al. 2012, Taylor et al. 2016). However, recorded dry matter yields for many of these species are likely too low to ensure feasible bioenergy production. Willow exhibited dry matter yields of 7.4 – 17.3 tons ha^{-1} yr^{-1}, Poplar produced 2.9 – 9.6 tons ha^{-1} yr^{-1}, while Sycamore and Sweetgum species managed to deliver only very low yields of 2.5 – 5.7 tons ha^{-1} yr^{-1} (Aylott et al. 2008, Hinchee et al. 2011). These dry matter yields are generally much lower than those reported across the majority of the grass species in this literature review.

Jatropha is a woody species highly regarded not only for its seed oil content, but also the characteristics of the oil which make its production and refinery into biodiesel very environmentally friendly and inexpensive. However, according to Pan and Xu (2011), the yield potential of jatropha is so low that as a diesel alternative this species is actually completely insufficient. Reinhardt et al. (2008) reported a yield of 1.6 - 4.9 tons ha^{-1} yr^{-1} dried seed, which equated to 0.530 – 1.790 l ha^{-1} yr^{-1}. Even though the characteristics of jatropha oil are highly favourable, the yield is simply just not on par with species such as alga, with a potential oil yield of 197 600 l ha^{-1} yr^{-1}.

Eucalyptus might be the species which offers the greatest feasibility of the woody species list above, with a potential dry matter yield of up to 27.5 tons ha^{-1} yr^{-1}. Sims et al. (1999), however, reported relatively larger dry matter yields for several popular woody species of up to 34 tons ha^{-1} yr^{-1}. Regretably, even these yields are generally much lower than those produced by the majority of the listed energy grasses.

According to Telmo and Lousada (2011), calorific values for pellets made from softwood species ranged between 19.7 - 20.4 MJ kg^{-1} and hardwood species between 17.6 - 20.8 MJ kg^{-1}. It should be noted that at best these values are perhaps only slightly greater than those achieved by energy grasses. Annual energy production should thus be expected to range between 44 - 707.2 GJ ha^{-1}. A vague but still rather poor WUE range of between 5.97 - 12.3 kg ha^{-1} mm^{-1} for popular woody species (Olbrich et al. 1993) might infer energy production at very high water requirements. The LAI of woody species seems to be a parameter which is not regularly analysed, however, the LAI values of *Eucalyptus* and Poplar are reported to be in the region of 5.7 m^2 m^{-2} and 0.6 – 4.4 m^2 m^{-2}, respectively (Myers et al. 1996, Fang et al. 1999).

2.8.2.5 Aquatic species

Aquatic plants such as the common reed (*Phragmites australis*) and water hyacinth (*Eichhornia spp*) also show potential for bioenergy production. The common reed has additionally proven to be effective in anchoring of coastal sands and soils and also in the absorption of fertilizer run-off from inland farms (Stankus 2014).

Knoll et al. (2012) found giant reed (*Arundo donax* L.) to be the least productive of his trial species, with a mere dry matter yield of 6.4 tons ha^{-1} yr^{-1}. However, well fertilized soils have delivered yields greater than 37 tons ha^{-1} yr^{-1} (Angelini et al. 2005). An average CV of 17 MJ kg^{-1} (Angelini et al. 2005) can unfortunately not make up for the rather low dry matter yields and thus results in a somewhat mediocre energy production of 629 GJ ha^{-1} yr^{-1}. These yields could also not even be redeemed by an impressive WUE because this value too was only in the range of 24.7 kg DM ha^{-1} mm^{-1} (Erickson et al. 2012). At first glance, an equal to slightly better than average seasonal water use of 1100 mm (Triana et al. 2015) provides a little encouragement, however, perspective is gained when low water use is paired with rather low biomass yields.

The poor WUE and average water use are rather surprising, because of the three species tested by Erickson et al. (2012), at 1m depth giant reed had a root biomass of 3.8 tons ha^{-1}, which was 1.3 and 2 times greater than that of napier and energy cane, respectively. From a sustainablity point of view such an enormous root biomass may potentially make up for areas which were found lacking because giant reed would likely be able to store more carbon within the soil than many other grass species.

An aspect which often offers a fair degree of possible improvement is LAI, however, giant reed already has a fairly high LAI of 6 - 7 m^{2} m^{-2} (Triana et al. 2011). The assumption might therefore be made that the likelihood of improving upon the potential of giant reed as a bioenergy species is rather limited. However, a high LAI is not necessarily always an advantageous trait because it generally suggests high water use (Grier and Running 1977). Furthermore, the higher the LAI of a grass species the greater its leaf to stem ratio would naturally be. As stated earlier, the greatest proportion of sugarcane’s mass is situated in the plant stems. Therefore, selective breeding of giant reed to improve its stem to leaf ratio might in fact be an area which could be improved upon with the hope of bettering the bioenergy potential of this species.

From a net energy perspective giant reed might at first glance seem extremely inadequate as a bioenergy grass because it simply cannot deliver the type of energy yields seen in many C4 species. Effectively, twice the amount of water or land would be required to yield the same amount of energy as napier (Erickson et al. 2012). However, the application of giant reed as an energy species is a practical example of fitting the most ideal species to a specific set of circumstances or environment. For instance, regions which are often flooded or even permanently saturated will pose an obstacle which cannot be overcome by species which are more sensitive to saturated soils. In such circumstances giant reed would be a species capable of filling a niche which would be the downfall of most other species.

2.8.2.6 Algae

Single-celled algae are able to divide and double their cell count every few hours and therefore are the plant family credited with the most rapid biomass accumulation known to man. Per hectare algae can produce 40 times more ethanol than maize, and can also be altered or modified to produce virtually all of the remaining fuel types in current use. Algae can also be cultivated in conjunction with coal power plants and perhaps throughout the majority of the industrial sector in order to scrub CO_2 and even more noxious GHG emissions (Schubert 2006).

An oil content range of between 15 – 77% for different algal species was established by Chisti (2007). Such potentially high oil concentrations have a large part to play in the equally impressive calorific values of between 18.6 - 28 MJ kg^{-1} of different algae apecies (Scragg et al. 2002, Phukan et al. 2011). With regards to potential yields, it seems that for algae the generally accepted approach is to refer to annual oil production rather than biomass or dry matter production. The enormous potential oil production of up to 197 600 l ha^{-1} yr^{-1}, if crudely converted can result in dry matter yields of almost 400 ton ha^{-1} yr^{-1}, far greater than any terrestrial flora species (Demirbas and Demirbas 2011). The resultant energy production of 7440 - 11 200 GJ ha^{-1} yr^{-1} is at the maximum end of its range, at least a single order of magnitude greater than the next best bioenergy feedstock.

It must, however, be duly noted that the start-up cost of algae bioenergy production is generally much greater than that of terrestrial crops, in addition to the fact that water requirements are much greater than for conventional crops (Wijffels and Barbosa 2010).

2.9 Species assessed in this study

There is a very definite advantage in using perennials rather than annuals in long-term energy production, and it is evident in the fact that the majority of the above-mentioned crops are indeed perennials. According to Knoll et al. (2012) grasses especially show great potential due to the fact that they produce substantial yields even under residual soil fertility and dryland conditions.

The potentially high establishment costs of certain perennials which are dependent on vegetative propagation may be deterring, but in the long run, savings regarding the discontinuation of yearly replanting should bring security and peace-of-mind. This does not then even take into account the likely reductions in soil, nutrient and water losses. However, some of these species have high nutrient removal rates and therefore soil productivity may decline after numerous years of cultivation without nutrient supplementation. Therefore, effective management will be required if high yields are to be consistently and sustainably repeated (Knoll et al. 2012).

The purpose of the information which follows is to compare the data acquired on the species used in the present trial with each other as well as with the species mentioned above. Due note must be taken that available information is sometimes fragmented or incomplete. Also, species were not all tested under the same procedures or environmental conditions and thus the information supplied about any particular species is not necessarily what should be considered as the general behaviour or performance of that species.

2.9.1 First generation feedstocks

2.9.1.1 Grain sorghum *(Sorghum bicolor L)*

Information on biomass production of grain sorghum is either lacking or incomplete. A grain yield of 7.2 tons ha^{-1} yr^{-1} was reported by Muchow (1988), however biomass production, and thus also WUE values, will need to be established by additional research. Grain sorghum does, however, comfortably claim the lowest water use of all crops in this review, with an exceptionally low 375 - 618 mm per season (Tolk and Howell 2003). Furthermore, grain sorghum is also reported to be nutrient use efficient, attributed to the efficient use of any nitrogen already available in the soil (Stankus 2014).

On a grain yield basis grain sorghum varieties produce very similar ethanol yields to maize, with the benefit of using less water and more effectively fending off water stress. Certain American ethanol production plants which are situated in close proximity to sorghum regions have even switched to sorghum as their primary feedstock source (Rooney et al. 2007). A very low LAI of 1.92 m^{2} m^{-2} (Garrity et al. 1984) is perhaps a major weak point, but at the same time a trait open to immediate attention and thus a source of hope for improved proficiency of this species.

Data regarding dry matter yields and calorific value could not be found. Proper analysis of these traits might unveil the true potential of this species and ensure complete and full exploitation of this crop in the future.

2.9.1.2 Sweet sorghum *(Sorghum bicolor)*

Sweet sorghum has the unmatched flexibility of being able to yield large quantities of cellulose, starch and even soluble sugars already in a fermentable form (Rooney et al. 2007). Additionally, research has suggested that sweet sorghum has the potential to outperform average sugarcane ethanol production by 30% per hectare. The reason for this being the 90% fermentation efficiency boasted by sweet sorghum, as well as the presence of reducing sugars which inhibit crystallization during ethanol production (Ratnavathi et al. 2003).

The by-products attained by sweet sorghum present great value-adding potential and will enhance the feasibility of this crop as a bioenergy feedstock. Both by-products, grain and bagasse, are of particular significance because they can be redirected towards the animal feed sector, and from a land use efficiency perspective this just adds even further to the flexibility of sweet sorghum. Reddy et al. (2005) found that a fairly high grain yield, especially for a by-product, of up to 6 tons ha^{-1} yr^{-1} can be produced and utilized as animal feed. Also, similarly to sugarcane, after the sugary plant juice has been extracted a solid lignin rich residue, or bagasse, remains.

Furthermore, sweet sorghum can tolerate an extensive array of environmental conditions, requires relatively low inputs and is drought tolerant (Guigou et al. 2011). Sorghum's drought hardiness is actually of such a nature that hot and dry regions such as the tropics and sub-tropics are often associated with sorghum production (Rooney et al. 2007). Put into perspective, this equates to water requirements and cultivation costs for sorghum being four and three times lower, respectively, than that of sugarcane production (Reddy et al. 2005). As a matter of fact, the water use of sweet sorghum at an extremely low 360 – 457 mm per season is the lowest seasonal water use of all the species in this literature review for which water use data was found (Ramos et al. 2012). These values are very similar to those attained by (Mengistu et al. 2016), at 391 – 436 mm per five month growth cycle, thus substantiating these claims.

Many different varieties of sweet sorghum exist and so, many different end uses can be begotten (Rooney et al. 2007). The diversity of the gene base means that selection and breeding can be done to increase crop cycles and so lengthen harvest periods, all while producing cultivars which are best suited to each region. If managed correctly, the harvest period of sweet sorghum could be extended past that of sugarcane and, therefore, if produced in conjunction with each other they would increase the length of time that feedstock is available for processing (Guigou et al. 2011). According to Reddy et al. (2005), the incorporation of sweet sorghum to certain sugarcane based bioethanol distilleries would greatly alleviate feedstock shortages experienced by these distilleries and aid in increasing current production efficiencies from the mere 50% presently achieved. The improved availability of feedstock throughout the year would support a "just-in-time" harvesting and delivery system and therefore would reduce or eliminate the need for costly, high volume storage. Such management and production gains can be pushed even further by cultivating genotypes which have a strong ratooning ability.

The desire of sustainability in bioenergy production favours perennial species for their generally cheaper long-term production and greater friendliness towards the environment. However, a valid statement made by Rooney et al. (2007) is that annual bioenergy crops will be essential as rotation crops in food production. These are important niches which need to be filled as efficiently as possible, and annuals allow for greater plasticity in demand as well as in production alterations.

Under South African climatic conditions average dry matter yields were reported at 35 tons ha^{-1} yr^{-1} (Olivier et al. 2015), however, much lower dry matter yields of between 7.3 – 18.1 tons per hectare per five month growth season were reported by Mengistu et al. (2016). A CV similar to that of sugarcane, at 17.4 MJ kg^{-1} would thus infer energy yields between 127 - 609 GJ ha^{-1} yr^{-1}, which is unfortunately all but exeptional. Mengistu et al. (2016) attained relatively mediocre WUE values of 18.5 – 44.7 kg DM ha^{-1} mm^{-1}, however, the higher WUE value of 64.3 kg DM ha^{-1} mm^{-1} reported by Olivier et al. (2015), coupled with an extremely high ethanol conversion efficiency puts a slightly different dynamic on sweet sorghum as a bioenergy crop.

2.9.2 Second generation feedstocks

2.9.2.1 Napier *(Pennisetum purpureum)*

Napier is able to fix a reasonable amount of nitrogen from the atmosphere and so improve soil quality and reduce required nitrogen supplementation. Where manure is added to the soil, napier also absorbs the greatest fraction of organic phosphorous as compared to other energy grasses. Attributes such as those mentioned above obviously reduce the significance of napier’s nutrient removal rates. However, due to its high biomass production, quantitatively it still removes high volumes of nutrients from the soil. Oddly enough, even this apparent weak point could be used advantageously by planting napier as a buffer around fields being intensely fertilized (Mayer et al. 2007).

The rapid establishment of napier could result in peak yields being achieved within the first year, whereas other species such as giant reed and switchgrass may require two or three years to achieve peak yields (Knoll et al. 2012). According to the research done by Knoll et al. (2012) on eight energy grass species, napier and energy cane showed the greatest yield potential throughout the trial. Khairani et al. (2013) found dry matter yields to vary between 13.4 and 33.5 tons ha^{-1} yr^{-1}, whereas Rengsirikul et al. (2013) attained dry matter yields of 75 tons ha^{-1} yr^{-1}. However, according to Knoll et al. (2012), napier experienced significant yield declines in the third and fourth year of their trial, probably attributed to nutrient depletion of the soil. Supplementation with organic matter such as biochar and digestate might therefore be fundamental in maintaining high and consistent yields over long-term production. However, a contradicting statement made by Knoll et al. (2012) in the same article is that even under conditions of low soil fertility, napier is able to produce substantial biomass yields. Woodard and Prine (1993) seem to agree with this statement after establishing that yields produced under low-input production systems were apparently not drastically lower than those recorded from well-irrigated and fertilized trials.

Cultivars presently in use have been selected for great leaf biomass, less fibre and greater nitrogen concentrations in order to produce better quality animal feeds. The selection for such attributes often leads to dry matter yields being sacrificed for the sake of producing better quality and improved palatability (Rengsirikul et al. 2013). Specific selection and conventional breeding for increased stem production and fibre content will thus most likely lead to even greater dry matter yields.

A calorific value of 16.4 MJ kg^{-1}(Rengsirikul et al. 2013), suggests an energy yield of 548 - 1227 GJ ha^{-1} yr^{-1}, and that at a fairly decent WUE of 52.4 kg DM ha^{-1} mm^{-1} (Olivier et al. 2015). The large yield capacity of napier is most certainly greatly accredited to an enormous LAI of up to 15.4 m^{2} m^{-2} (Kubota et al. 1994).

According to Knoll et al. (2012), during a trial conducted on both napier and sugarcane, napier used 17% less water than conventional sugarcane. The results attained by Olivier et al. (2015) claim a seasonal water use of 962 - 1184 mm and thus support Knoll's findings.

Unfortunately, on the other hand, napier's establishment could turn out to be rather costly due to the fact that it needs to be propagated vegetatively (Jank et al. 2013). Also, a drawback of napier is that it exhibits traits of invasiveness and therefore strict management must be implemented in order to keep a stand from becoming threatening to local ecosystems (Stankus 2014). Despite these drawbacks Erickson et al. (2012) tend to agree with the fact that napier shows great potential as a second generation bioenergy crop.

2.9.2.2 Miscanthus *(Miscanthus giganteus)*

Miscanthus has been shown to produce 2.5 times more ethanol than existing maize-based ethanol operations at 10% lower cost requirements of switchgrass production. Also, with regards to second generation grasses, miscanthus is rated as the most water use efficient grass under irrigation (Stankus 2014), and has attained WUE values as high as 90 kg DM ha^{-1} mm^{-1} (Erickson et al. 2012).

Mature miscanthus stands under dryland conditions averaged 16 tons ha^{-1} yr^{-1} with a maximum of 37 tons ha^{-1} yr^{-1} dry matter yield (McLaughlin and Walsh 1998). These yields are very similar to those attained by Waclawovsky et al. (2010), reporting dry matter yields of 29.6 tons ha^{-1} yr^{-1}. With a CV of 16.5 MJ kg^{-1} (Ercoli et al. 1999), a total energy production of between 264 - 610.5 GJ ha^{-1} yr^{-1} can be expected. Species such as napier and sugarcane can produce virtually double these energy yields, however, at a WUE of roughly only 58% and 67%, respectively, of that of miscanthus. Besides the two sorghum species covered in this review, miscanthus is reported to have the lowest seasonal water use of all the included species, at 900 mm (Triana et al. 2015). However, if the WUE of miscanthus is calculated using the maximum yield proposed by McLaughlin and Walsh (1998) and the seasonal WU reported by Triana et al. (2015), then a WUE of only 41 kg DM ha^{-1} mm^{-1} is attained. This calculated WUE is less than 50% of that claimed by Erickson et al. (2012) and thus questions the validity of his claim. If this calculation is correct it would also mean that miscanthus is no longer the most water use efficient species of this review, but actually the seventh most water use efficient.

According to Pinners (2014), miscanthus exhibits virtually all the attributes necessary for a high potential energy crop, and the extremely high LAI of 6 - 12 m^{2} m^{-2} merely supports these claims (Hastings et al. 2009). However, the data around miscanthus needs to be validated before conclusions can be drawn about the potential of this species.

2.9.2.3 Vetiver *(Chrysopogon zizanoides)*

This grass may possibly be more effective at sequestering and storing carbon than any other *Poaceae* species in current cultivation. Vetiver can also withstand a wide range of climatic variations; from tropical to sub-temperate, drought, and even complete soil saturation for up to three months (Truong 2003). What's more, fresh leaves are palatable, which allows for even greater flexibility in utilization (Pinners 2014). The range of positive attributes set forward by vetiver is particularly valuable because they add greatly to the utility and ability of the species to occupy niches which would otherwise perhaps be left barren. If sustainability is to be taken seriously, then such attributes may carry the same weight as high yields or high water use efficiency.

Well-established vetiver hedges have been found to reduce soil erosion exceptionally well and simultaneously improve water infiltration and thereby recharge underground water banks (Truong and Loch 2004). Preventing erosion is extremely important, not only to maintain soil health, but also to keep carbon which has been deposited in the soil in place. Also, vetiver stands have been known to stay productive for more than 20 years with fertilizer supplementation. The environmental, financial and sustainability advantages of such a long lifespan are indispensable.

Vetiver is extremely hardy and thus can be used to revegetate soils which offer non-ideal growth conditions such as coastal acid sulphate soils, erosion prone soils, waste dumps, shifting dunes, saline soils and also degraded laterite soils (Xu 2002). Vetiver has successfully been used before to rehabilitate saline soils and regain soil quality in order to revert back to other land uses. It has even been used in applications of treating and filtering waste water by growing the grass within floating frames on top of the affected body of water (Boonsong and Chansiri 2008).

A practical example of vetiver used in the industry is that of a 50 MW electricity plant in Baraona, Dominican Republic. This power plant requires 900 tons of dried vetiver per day, which is harvested from 12.5 hectares of land. This equates to just over 4500 hectares required to run the production plant year round. According to the financial estimations of this specific electricity plant, it is more than 50% cheaper to use vetiver instead of coal to power the plant (Pinners 2014).

When Pinners (2014) planted vetiver at a spacing of 0.3 m x 0.3 m on reasonably deep and fertile soils, dry matter yields of 120 tons ha^{-1} yr^{-1} were recorded, and under poorer conditions a still very impressive yield of 70 tons ha^{-1} yr^{-1} was attained. These massive biomass yields are most certainly greatly attributed to vetiver's enormous LAI of 14 m^{2} m^{-2} (Truong 2008). The massive dry matter yield and LAI are most likely due to the extremely large root system of the species (Pinners 2014). With such high dry matter yields, and a CV of 16.3 MJ kg^{-1}, energy yields are expected to range between 1141 - 1956 GJ ha^{-1} yr^{-1}. This is comfortably the highest energy yield of any terrestrial species covered in this review.

Unfortunately, little to no information was attained with regards to water use and water use efficiency and therefore it is currently not possible to give a full account of the feasibility of a species which seems to have proven its potential in all other categories.

2.9.2.4 Guinea grass *(Panicum maximum)*

As mentioned previously, the establishment of napier might be rather costly due to the fact that napier needs to be propagated and planted vegetatively. A seeded species which might be a viable alternative to napier is guinea grass. According to Jank et al. (2013), dry matter yields vary from 24.1 tons ha^{-1} yr^{-1} under minimal fertilization, to 49.1 tons ha^{-1} yr^{-1} under fair fertilization. The 10 different guinea grass species tested by Grof (1970) yielded similar dry matter yields of between 25.5 - 44.7 tons ha^{-1} yr^{-1}.

This species is however chiefly considered to be a forage species, and though this feature adds to the versatility of guinea grass, it also unfortunately means that data is lacking, especially with respect to the bioenergy aspect of the species. Most data available on water use, WUE, CV or LAI is thus reflective of guinea grass under forage conditions and management and is thus not indicative of its bioenergy potential. However, available data is presented simply to give an idea of what values could be linked to this species.

Pieterse et al. (1997) attained water use efficiency values of between 16.1 – 28.2 kg DM ha^{-1} mm^{-1} for four different guinea grass cultivars under different rates of nitrogen fertilization. Under forage conditions, certain *P. maximum* cultivars produced LAI values greater than 6 m^{2} m^{-2} within only 30 days (Singh et al. 1995). It must, however, be kept in mind that these cultivars have been specifically bred for “leafiness” in order to improve feed yield and quality. Breeding guinea grass for biomass production could lead to greater stem to leaf ratios and thus a decline in the above-mentioned LAI values (Mulkey et al. 2006). Water use and CV data do not seem to be available for this species.

It is obviously essential to have as large a base of primary bioenergy species as possible and thus if data is lacking with regards to a prospective species, then these deficient areas need to be stringently addressed through research.

2.9.2.5 Brazilian grass *(Brachiaria brizantha)*

Brachiaria brizantha is also a common forage grass and so very little information is also available on this species as a biomass or bioenergy crop. Data available was thus obtained from sources where the scope of research done was focused on Brazilian grass only under forage management. Dry matter yield was therefore not determined annually, but rather at the end of a single six week cycle. It is therefore difficult to determine or estimate the potential of this grass on a biomass basis. Nevertheless, Guenni et al. (2005) states that Brazilian grass attained dry matter yields of 2.8 - 3.5 tons ha^{-1} at the end of the six-week growth period. If it is assumed that six such six-week growth cycles are available per annum, then a crude dry matter yield of 16.8 – 21 tons ha^{-1} yr^{-1} could be achievable.

At the end of the relatively short six-week trial period a rather unexpectedly large LAI of 4 m^{2} m^{-2} had been produced (Meirelles et al. 2011). It would be expected that biomass production would increase and LAI would decrease if this species was to be cultivated specifically with the goal of producing bioenergy instead of forage.

An admirable trait of Brazilian grass is that it is particularly well adapted to soils which are considered to be nitrogen restrictive (Guenni et al. 2005). As a result, poorer quality soils might not be as much of a hindrance to Brazilian grass as compared to other species which have high nutrient removal rates. Such resourceful traits are always welcome on the sustainable bioenergy front, especially when scales are extrapolated to a global magnitude.

2.9.2.6 Blue thatch grass *(Hyparrhenia tamba)*

Blue thatch grass is a species on which no data could be found with respect to biomass production, pasture cultivation or bioenergy use. Data acquired via this trial would thus have to serve as the extent of information currently available to call judgement upon the bioenergy capacity of this species.

2.10 Species summary

Table 2.2 makes for easy and rapid comparison between the different species covered in this literature review. It not only assists in identifying the most promising traits of different species, but also allows for the identification of areas which can be improved upon in order to gain the most from each species.

Table 2.2: Values of leaf area index (LAI), dry matter yield (DMY), water use (WU), water use efficiency (WUE), calorific value (CV) and energy yield (EY) of several popular bioenergy species as reported in available literature (Garrity et al. 1984, Muchow 1988, Patil et al. 1988, Stout 1992, Olbrich et al. 1993, Kubota et al. 1994, Myers et al. 1996, Pieterse et al. 1997, Teruel et al. 1997, Madakadze et al. 1998, McLaughlin and Walsh 1998, Ercoli et al. 1999, Fang et al. 1999, Sims et al. 1999, Tolk and Howell 2003, Guenni et al. 2005, McLaughlin and Kszos 2005, Sridhar et al. 2007, Schmer et al. 2008, Truong 2008, Botha 2009, Hastings et al. 2009, Hong et al. 2011, Telmo and Lousada 2011, Triana et al. 2011, Erickson et al. 2012, Knoll et al. 2012, Ramos et al. 2012, Jank et al. 2013, Rengsirikul et al. 2013, Pinners 2014, Sadaka et al. 2014, Na et al. 2015, Olivier et al. 2015, Triana et al. 2015, Mengistu et al. 2016).

	Parameter					
	LAI	**DMY**	**WU**	**WUE**	**CV**	**EY**
Species	m^{2} m^{-2}	tons ha^{-1} yr^{-1}	mm	kg DM ha^{-1} mm^{-1}	MJ kg^{-1}	GJ ha^{-1} yr^{-1}
Switchgrass	6.1 – 8	5.2 – 11.1	?	25 – 85	18 – 19	93.6 – 210.9
Sugarcane	6 – 7	39 – 85	975 – 1100	60	17.4	678.6 – 1479
Energy cane	4 – 5	20 – 70	1629	45.4	~17.4	348 – 1218
Bamboo	4.8	49.5	?	?	17.6	871.2
Tree species	~0.6 – 5.7	12 – 34	?	5.97 – 12.3	19.7 – 20.8	211.2 – 707.2
Giant reed	6 – 7	37	1100	24.7	17	629
Algae	-	400	-	-	18.6 – 28	7440 – 11 200
Napier	15.4	33.5 – 75	962 – 1184	52.4	16.4	548 – 1227
Grain sorghum	1.9	~7.2	375 – 618	?	?	?
Sweet sorghum	?	35	360 – 457	64.3	17.4	609
Miscanthus	6 – 12	16 – 37	900	90	16.5	264 – 610.5
Vetiver	14	70 – 120	?	?	16.3	1141 – 1956
Guinea grass	?	24.1 – 49.1	?	16.1 – 28.2	?	?
Brazilian grass	?	~ 16.8 – 21	?	?	?	?
Blue thatch grass	?	?	?	?	?	?

CHAPTER 3

MATERIALS AND METHODS

3.1 Site specifications and information

The trial took place on the Hatfield Experimental Farm of the University of Pretoria, South Africa. The site is located at coordinates 25° 45' S and 28° 16' E and is at an altitude of 1327 m above sea level. This is a summer rainfall region which receives an average annual rainfall of 670 mm. The soil is a Hutton with clay content ranging between 26 – 37 % and the pH in water is between 6.2 – 6.7 (Tesfamariam et al. 2010). An on-site weather station recorded and logged daily weather for the duration of the trial.

3.2 Trial design and layout

The trial was laid out as a two-factorial split-plot randomised block design with three water regimes and eight *Poaceae* species; (the ninth species, *Cymbopogon excavatus* (S3 - Bushveld turpentine grass), was eventually omitted from the trial due to extremely poor establishment). The trial lasted from the beginning of December 2014 until the end of November 2015. Treatments were randomly allocated to plots within each of three replicate blocks. The trial consisted of a total of 81 plots; each measuring 5.5 x 6 m.

Figure 3.1 represent the layout of the treatment plots within each replicate.

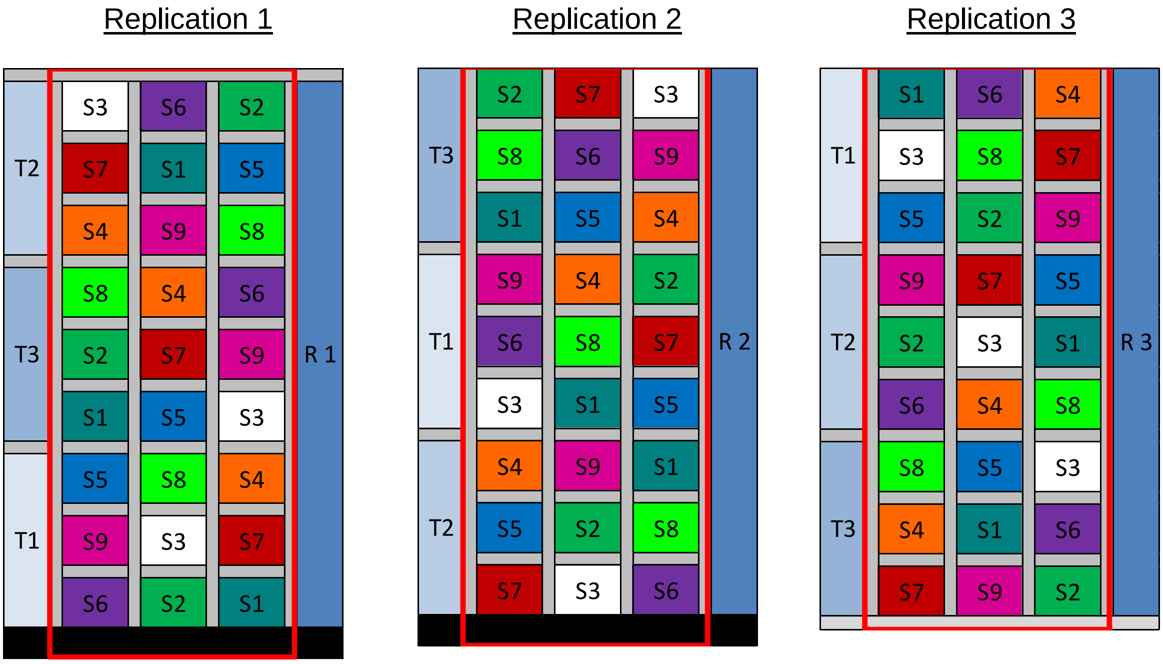
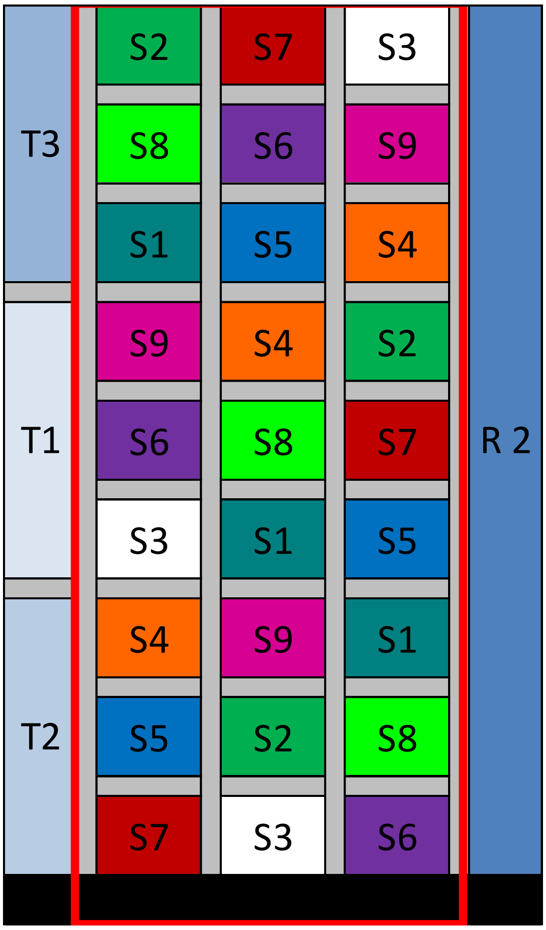


Figure 3.1: An illustration of the layout of the field trial with respect to the random allocation of species (S) within water treatments and the random allocation of water treatments (T) to replicated blocks (R). See Figure 3.2 for legend.



Legend

- “S” – Species (x9)
- “T” – Water treatments (x3)
- “R” – Replicate (x3)
- Plots – 5.5 x 6 m
- Trial layout – 3 plots horizontally x 27 plots vertically
- Trial dimensions – 19 m horizontally x 167 m vertically
- Horizontal and vertical grey columns – 1 m spacing between plots
- Black border – 3 m separation between “R”
- “T” and “R” Side bars – serve merely as illustrations and do not form part of trial dimensions

Figure 3.2: Description of field trial labels.

3.3 Selected trial species

Photos of the eight *Poaceae* species that were included in the trial are shown in the figures below:

Pennisetum purpureum

(S2 Napier – I)

Miscanthus giganteus

(*S4* Miscanthus – E)

Chrysopogon zizanoides

(S5 Vetiver – E)

Hyparrhenia tamba

(S6 Blue thatch – I)

Figure 3.3: Vegetatively propagated species. The “I” or “E” which follows on the common name indicates whether the species are indigenous or exotic to South Africa, respectively.

Panicum maximum

(S1 Guinea grass – I)

Brachiaria brizantha

(S7 Brazilian grass – I)

Sorghum bicolor

(S8 Sweet sorghum – I)

Sorghum bicolor

(S9 Grain sorghum – I)

Figure 3.4: Seeded species. The “I” or “E” which follows on the common name indicates whether the species is indigenous or exotic to South Africa, respectively.

3.4 Soil preparation

The site was prepared by ploughing to loosen the soil, followed by several repetitions of discing to give a fine tilth. Thereafter plots, borders, walkways and paths were measured out and marked off. Augers were used to drill the holes needed to insert aluminium neutron probe access tubes necessary for volumetric soil water content measurements. The access tubes were 1.2 m in length and inserted to a depth of 1.1 m.

3.5 Planting and fertilization

The term “cycle” refers to three successive monthly harvest intervals, and is also the length of time allotted until the final harvest. The first cycle took place from beginning December 2014 to end February 2015, cycle two from beginning March 2015 to end May 2015 and cycle three from beginning September 2015 to end November 2015.

In the case where species were to be planted vegetatively, spades and augers were used to prepare holes of appropriate depth and size, generally 25 x 25 x 25 cm each. Each hole was dressed with 20 g Haifa Multicote NPKS (15:3:12:7) fertilizer just prior to planting. Where planting was done by seed, the same quantity of fertiliser (400 kg ha^{-1}, according to soil analysis) was broadcast over each plot and then rotovated into the soil just before planting or sowing.

Fertilizer was, however, not applied before the second cycle due to the expectation of low nutrient removal rates generally experienced during the colder and shorter days of autumn (Roncucci et al. 2015). In August 2015, just before the beginning of the third cycle, the same amount of fertilizer was applied to each plot for the second time. Shallow furrows were made 10 cm from and parallel to the rows of species planted vegetatively and the fertilizer distributed evenly among each furrow. Fertilizer was applied in this method rather than broadcast in an attempt to do as little damage to shallow roots as possible. For S1 and S7 the fertilizer for each plot was broadcast and then lightly worked into the soil using rakes. The sorghum plots (S8 and S9) were fertilized by means of broadcasting, followed once again by rotovation to loosen the soil and incorporate the fertilizer into the soil.

Napier (S2), S4, S5 and S6 were planted vegetatively at a spacing of 1 m between rows and 0.5 m within rows, resulting in 66 plants per plot. The species S4, S5 and S6 were planted in April 2014, while S2 was planted after the winter during September 2014. These are all perennial species and therefore were only planted once at the beginning of the trial.

Seeds of S1 and S7 were sown by broadcasting the seed during the second week of November 2014 at a rate of 7.5 kg ha^{-1}, 1.5 times the rate used by Abdi et al. (2015). Guinea grass (S1) and S7 have very fine seeds and therefore it was necessary to mix the specified weight of seed with fine sand to allow for more even distribution when broadcast by hand. These are also perennial species and therefore they were also planted only once at the beginning of the trial. After sowing, plots were lightly raked to cover the seed with soil and then rolled to ensure good soil to seed contact.

Sweet sorghum (S8) and S9 were planted at a stand density of 16 000 and 12 000 plants per hectare, respectively. These two species, being annuals, were planted just prior to the beginning of each cycle. A manual hand planter was used to plant rows spaced 1 m apart.

Thinning of sorghum rows was done by hand three to four weeks after planting, to a spacing of 6.25 and 8.33 cm between plants for S8 and S9, respectively.

Weeding was done regularly by hand at the beginning of each cycle, where after dense canopy cover of the test species generally inhibited the growth and germination of weeds.

3.6 Irrigation and soil water content measurements

For the first three weeks of each cycle, all planted or replanted species received overhead irrigation as required, in order to facilitate establishment. Thereafter irrigation was applied via an above-ground drip line system with an application rate of two litres per hour when water pressure was between 100 - 150 kPa. Drip lines were spaced 1 m apart.

The three different water treatment regimes were as follows: the first was a dryland water treatment (T1), the second, (T2), received 15 mm of supplementary irrigation (in addition to rainfall) every alternative week, and the third, (T3), received 15 mm of supplementary irrigation every week. Irrigation amounts were occasionally increased or skipped to prevent excessive stress, depending on the rainfall and soil water content measurements.

Water treatments T2 and T3 were not fully irrigated on purpose because of the fact that in South Africa the irrigation of bioenergy crops is not desirable (Department of Environmental Affairs). Therefore, water treatments T2 and T3 were included to simulate regions with higher rainfall than Pretoria, but still be representative of dryland production in South Africa.

A calibrated neutron probe was used to measure the volumetric soil water content (SWC) of each plot on a weekly basis for the duration of the trial. Measurements were taken at soil depths of 20, 40, 60, 80 and 100 cm. Weekly measurements of SWC together with rainfall and irrigation data was used to calculate the soil water blance and crop water use.

3.7 Harvesting and data sampling

At the start of December 2014 all vegetatively planted species were cut back to a height of roughly 5 cm, using brush cutters and pruning shears. Species which were planted by seed were then roughly cut to the same height. Beginning of December 2014 was therefore officially the beginning of cycle one and the start of the trial.

Harvesting, also considered to be destructive sampling, was done for one third of the plants in a plot at the end of each month for all three months of all three cycles (Figure 3.5). The dormancy experienced by sub-tropical species during the winter months, June - August, obviously lead to insignificant yields and thus these three months did not contribute to the annual potential yield.

Figure 3.5 illustrates the repeated harvest procedure for each plot throughout the three months of each cycle. As illustrated, borders were left around and between each sample area. The sample area was 1 x 4 m^2, which otherwise equated to eight individual plants within the same surface area for the vegetatively planted species.

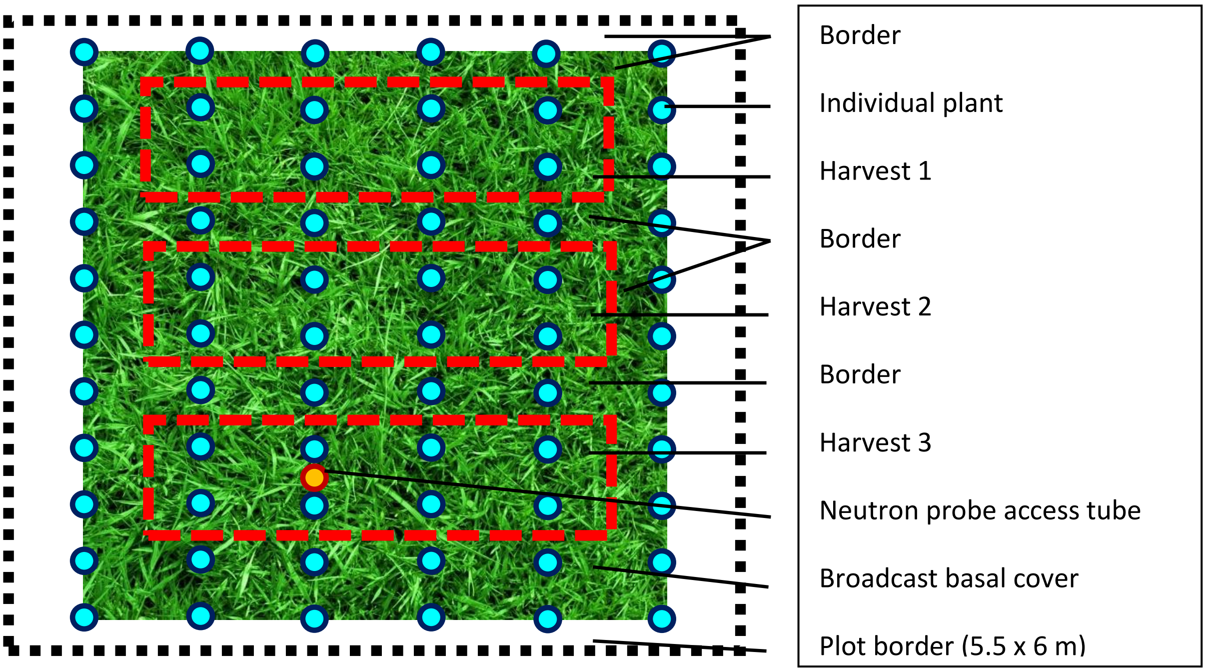


Figure 3.5: Illustration of plot dimensions, harvest areas and labels of the field trial.

Harvesting was done using brush cutters and pruning shears. All species were cut to a height of 5 cm, with the exception of S2, which was cut at 10 cm. This was because once S2 had been fairly well established, the bottom 5 cm of the bushes were too dense and too hard to allow for the 5 cm cut. Once the entire sample area of each plot was cut, it was weighed to determine the sample's fresh mass. A sub-sample was then taken and the mass recorded, where after the leaf area was measured using a LI 3100 leaf area meter. The sub-sample was then put into a paper bag and placed in an air-drying oven at 65 ^{o}C until a constant dry mass was achieved, where after this value was also recorded.

3.8 Water use and water use efficiency

Water use of each species within each cycle was determined by soil water balance calculations: Water use (ET) = Irrigation (I) + Precipitation (P) – Runoff (R) – Drainage (D) ± Storage (dS). Runoff was assumed to be zero due to the flat topography and absence of heavy down pours. Drainage was assumed to be negligible, as soil water content was monitored and irrigation carefully applied.

Water use efficiency was calculated by dividing the total dry matter production of each species within each water treatment by the total amount of water used for that specific harvest interval.

3.9 Calorific values

Due to budget constraints, as well as literature suggesting a fairly limited variation in range of grass calorific values (CV), the CV values were only analysed for the final harvest of cycle one. A sub-sample of the three replications of each water treatment and species was pooled for the analysis. Each of the 24 samples was then milled to pass through a 1 mm sieve, where after the CV value of each was determined using an oxygen bomb calorimeter.

3.10 Canopy cover

Ceptometer (Decagon Devices, Pullman, USA) readings were taken every week for the duration of the trial. Readings were taken in the part of the plots which would produce the final harvest. Unfortunately, due to unforeseen circumstances, readings were not taken for cycle one. However, full sets of readings were recorded for cycles two and three.

A single reading was taken above each plot and then five readings were taken at random at 20 cm height above the ground within each plot and then averaged. Fractional interception (FI) of photosynthetically active radiation (PAR) was determined by subtracting the ratio between the “below” and “above” readings from “1” : FI = 1 – (below / above). This data gave an indication of the growth rate and efficacy of canopy cover.

CHAPTER 4

RESULTS AND DISCUSSION

The end goal of this project was not only to determine the annual energy production of each grass species in question, but also to conclude how efficiently each species used water under different water regimes in the production of this energy.

Energy production, in this case, is the product of dry matter yield and calorific value per unit dry matter. However, since literature confirms that the calorific values of grass species have a very limited range of variation (Table 2.2), the bulk of the energy production capacity of each species thus depends primarily on annual dry matter yield. However, regardless of the fact that dry matter yield is probably the most important parameter under discussion, all parameters tested in this trial will be discussed in order to give a more complete view of the trial outcome.

An important factor to remember with regards to bioenergy production, and production of bioenergy crops in South Africa as a whole, is that South Africa is a water scarce country and the irrigation of bio-energy crops is undesirable (Department of Environmental Affairs). Thus, when determining which species produced the greatest yields and values, specific consideration should be kept in mind with regards to the results attained under the dryland conditions, especially in terms of dry matter production.

Statistically, many of the results attained for the present study were not as anticipated. High variance between replications resulted in large value variations being deemed significantly indifferent, not just with respect to fresh matter yield, but across all parameters. A likely cause of these results is that variation in soil quality and properties between the different replications may have been greater than anticipated. However, though this does present a challenge with regards to comparing or analysing results on a finer level, it does mean that where significant differences existed, it was on a grand scale.

4.1 Fresh matter yield

Please take note that the following abbreviations will be used throughout Chapter 4: C1 – Cycle 1 (1 Dec 2014 – 28 Feb 2015), C2 – Cycle 2 (1 Mar 2015 – 31 May 2015), C3 – Cycle 3 (1 Sep 2015 – 30 Nov 2015), T1 – Water Treatment 1 (dryland water treatment), T2 – Water Treatment 2 (15 mm supplementary irrigation every second week, depending on soil water content), T3 – Water Treatment 3 (15 mm supplementary irrigation every week, depending on soil water content), S – Species, S1 - *Panicum maximum* (Guinea grass), S2 - *Pennisteum purpureum* (Napier), S4 - *Miscanthus giganteus* (Miscanthus), S5 - *Chrysopogon zizanoides* (Vetiver), S6 - *Hyparrhenia tamba* (Blue thatch), S7 - *Brachiaria brizantha* (Brazilian grass), S8 - *Sorghum bicolor* (Sweet sorghum) and S9 - *Sorghum bicolor* (Grain sorghum).

In order to better understand the results which are to be discussed in the subsequent subsections, an understanding of the total rainfall and irrigation for each respective water treatment needs to be grasped. Therefore, the water application of the three different water regimes across the three production cycles is illustrated in Figure 4.1.

4.1.1 Production Cycle 1: Dec 2014 – Feb 2015

There were statistically significant differences between species and interactions (Table 4.1). Treatment combinations concerning S1 and S5 produced their highest yields at T2, followed by T3 and then T1. Treatment combinations of S4 and S6 exhibited differing results as compared to those of S1 and S5 in that the lowest yields were produced at T2, with the highest yields at T3, followed by T1. The treatment combinations of S1, S4, S5 and S6 thus exhibited generally unexpected results since they did not produce decreasing yields according to decreasing water levels. The treatment combinations of S9 exhibited virtually unvarying yields across all three water treatments. Only the treatment combinations of S2, S7 and S8 followed the generally expected trend of increased fresh matter production with increasing water application (Figure 4.2). Large variations between replications had a profound effect across all treatment combinations resulting in very large yield variations being significantly similar.

All treatment combinations of S4 (T1 - 4.30, T2 - 3.97 & T3 - 9.33 tons ha^{-1}), S5 (T1 - 21.55, T2 - 29.57 & T3 - 28.36 tons ha^{-1}) and S9 (T1 - 20.12, T2 - 20.13 & T3 - 19.84 tons ha^{-1}) produced lower yields than the average yield of cycle 1 (42.22 tons ha^{-1}) (Figure 4.2). Treatment combinations of S1 T1 (38.01 tons ha^{-1}), S6 T2 (30.07 tons ha^{-1}) and S7 T1 (30.57 tons ha^{-1}) also produced lower yields than the cycle average. All the above mentioned treatment combinations were significantly similar to each other (Table 4.1).

Only the treatment combinations of S2 (T1 - 60.87, T2 - 63.15 & T3 - 68.74 tons ha^{-1}) and S8 (T1 - 48.57, T2 - 58.70 & T3 - 63.17 tons ha^{-1}) produced yields which were greater than the cycle average across all water treatments. It was, however, the treatment combination of S1 at T2 (92.12 tons ha^{-1}) which produced the greatest yield of cycle 1. Despite relatively large yield variations many treatment combinations were significantly similar to S1 at T2, namely; S1 (T3 - 63.90 tons ha^{-1}), S2 (T1 - 60.87, T2 - 63.15 & T3 - 68.74 tons ha^{-1}), S6 (T1 - 51.26 & T3 - 57.72 tons ha^{-1}), S7 (T2 - 60.49 & T3 - 68.73 tons ha^{-1}) and S8 (T2 - 58.70 & T3 - 63.17 tons ha^{-1}) (Table 4.1).

Only the treatment ombinations of S2, S7 and S8 exhibited their greatest yields at water level T3, with gradually decreasing yields at T2 and then again at T1 (Figure 4.2).

The extremely high yield produced by S1 at T2 relative to both T1 and T3, was as a result of a very high yield generated in replication 1 of T2. It is uncertain what resulted in the production spike, however, by cycle 3 it is clear that the cause of the strangely high average had been phased out because yields then followed the expected trend of increased production according to increased water levels, as exhibited by treatment combinations of S2, S7 and S8 in Figure 4.2. Unless excessive amounts of water are applied, increased biomass production following increased water application is a commonly observed phenomenon, as was illustrated by the trials of Snowdon and Benson (1992).

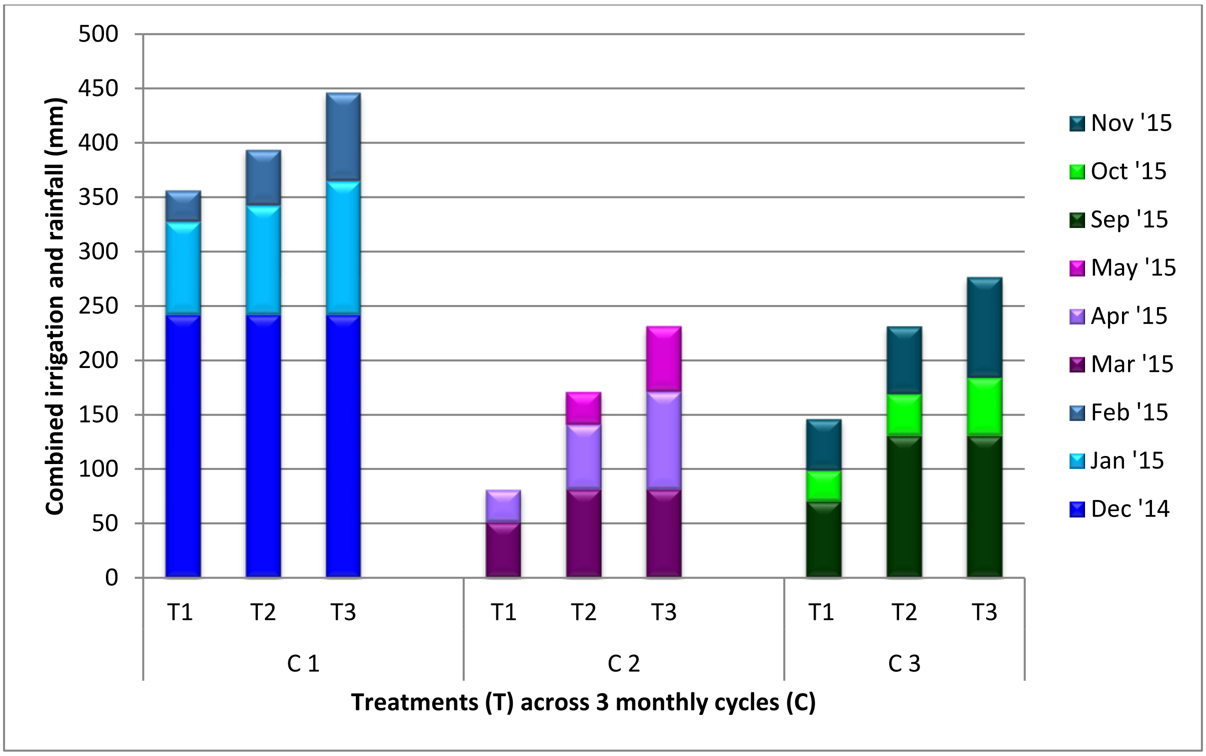


Figure 4.1: Combined 3 monthly rainfall and irrigation per water treatment (T1 – T3) of the three production cycles (C1 – C3).

The treatment combination of S5 at T2 produced a slightly greater yield as compared to S5 at T3, however, in this case the slightly higher yield at T2 was most likely attributed to a majority of smaller plant sizes, by chance, being planted in T3 plots. This effect is fortunately only witnessed in cycle 1. The reason that differing plant sizes are expected to have had attributed so definitely to unexpected results is because of the fact that certain species were first grown in bags before being transplanted into the field. For S4, S5 and S6 specifically, it was noted that severe variations in plant sizes had occurred within the bags, ranging from weak and timid plants to plants which had filled the entire capacity of the bag.

The treand of treatment combinations of S4 varied in all three cycles and also continually produced poor yields throughout each of these cycles. In the first cycle a relative drop in yield at T2 as compared to T1 and T3 was exhibited. A more detailed discussion with respect to the poor stand development and results of S4 will be given in section 4.6.

Since the effect on treatment combinations of S6 was also only exhibited in cycle 1, it is once again suspected that the cause can be attributed to weaker or smaller plants being planted in T2 plots, similarly to S5 (Figure 4.2).

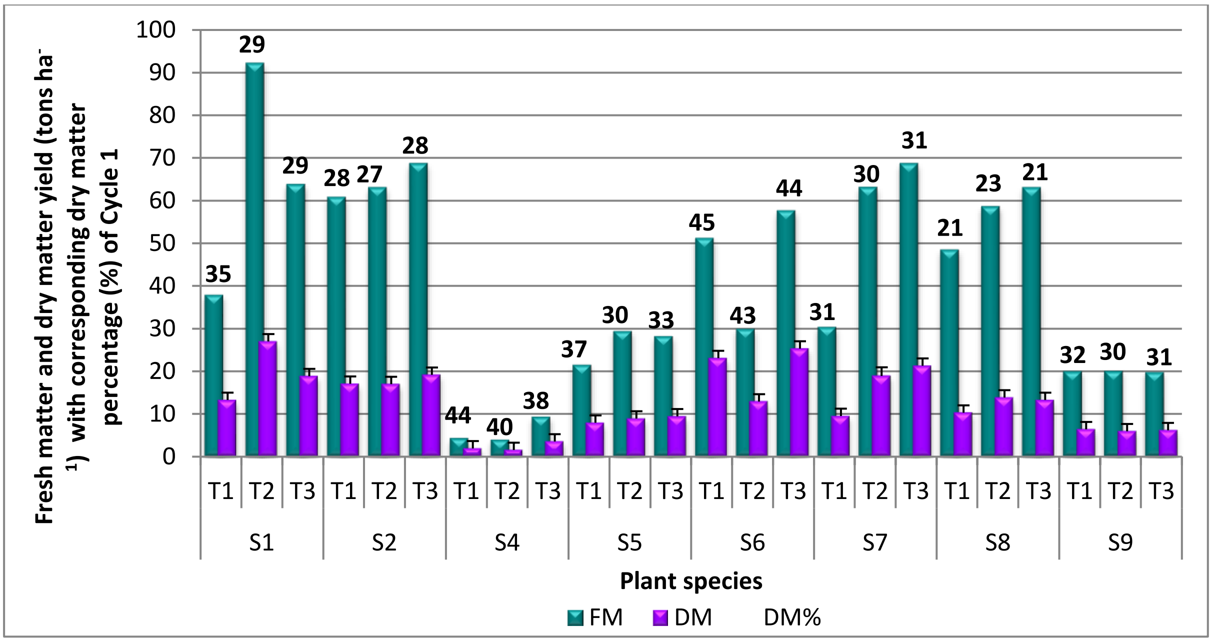


Figure 4.2: Fresh matter (FM) and dry matter (DM) yield with corresponding dry matter percentage (above FM bars – DM %) of eight potential bioenergy sub-tropical *Poaceae* species (S) under three different water treatments (T) from 1 Dec 2014 – 28 Feb 2015 (Cycle 1). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Only S9 produced treatment combinations which showed virtually no variation to water treatments (Figure 4.2). These results may, however, partly be due to birds feeding on the maturing seed heads of this species just prior to the final harvest. On the other hand, the selective breeding that sorghum has undergone in the aim of producing varieties which are extremely drought tolerant may form the foundation for a compelling alternative theory (Rooney et al. 2007). That is to say that grain sorghum has been selectively bred to the point where the water requirements of the species had in this case already been satisfied at T1. Therefore, any additional water would in actual fact be surplus and not result in increased yields.

As mentioned earlier in this chapter, the ability of any bioenergy species to produce substantial yields under dryland conditions will be a necessity if these crops are to be cultivated in South Africa. However, it must be kept in mind that different regions of South Africa will offer different rainfall levels, and thus different dryland conditions. Therefore, species should not be discredited as potential bioenergy crops if they do not perform well under the dryland conditions of this trial. Different regions might have rainfall levels equivalent to or even higher than water level T3 of this trial and thus supply the more water demanding species of this trial with the water requirements necessary to be highly productive. Another point which cannot be neglected is that different perennial species establish at different rates, and thus finite conclusions regarding the bioenergy potential of these species should not be drawn exclusively from first season data.

4.1.2 Production Cycle 2: 1 Mar 2015 – 31 May 2015

In this production cycle the interaction effects were not statistically significant, which implies that species responded similarly to different water regimes. However, the main effects of species and water treatments exhibited statistically significant differences (Table 4.2).

The mean yield produced at T2 (9.97 tons ha^{-1}) and T3 (10.05 tons ha^{-1}) did not differ significantly from each other, while that of T1 (5.12 tons ha^{-1}) was significantly lower than both of the supplementary irrigation treatments (Table 4.2).

From Figure 4.3 it is clearly evident that fresh matter yields for cycle 2 were generally drastically lower than those reported for cycle 1 (Figure 4.2). The average across all species in cycle 2 was only 8.38 tons ha^{-1}, a mere 20% of the average attained in Cycle 1. However, lower yields were definitely to be expected in cycle 2 as a result of seasonal weather changes from summer to autumn (Sinclair et al. 2001). Day lengths were becoming shorter, maximum and minimum air temperatures were on a decline and rainfall also decreased drastically (Figure 4.4).

With the exception of S1, which produced its greatest yield at T2, and S2, which followed the same trend, all other species performed as expected and produced increasing yields with an increase in the amount of water applied (Figure 4.3).

The three greatest yields were generated by only two species (Figure 4.3); S7 (T3 – 23.15 tons ha^{-1}; T2 – 18.91 tons ha^{-1}) and S1 (T2 – 18.59 tons ha^{-1}). The third best performing species, S2 (T2 – 14.04 tons ha^{-1}), lies in fifth place overall, behind a once again featuring, S7 (T1 – 14.33 tons ha^{-1}). These three species were also the only species able to produce yields greater than the average across all species, (8.38 tons ha^{-1}). The lowest yield was produced by S4 (T3 - 1.706 tons ha^{-1}), followed by S8 (T3 - 4.241 tons ha^{-1}) and S5 (T3 - 10.11 tons ha^{-1}) (Figure 4.3).

Since S7 produced three of the top four yields, it is not surprising that the mean yield of this species (18.80 tons ha^{-1}) was significantly greater than all other species (Table 4.2). Thereafter, however, very little significant differences existed between all remaining mean yields. The second and third best performing species, S1 (11.36 tons ha^{-1}) and S2 (10.51 tons ha^{-1}), were not significantly different from S5 (8.04 tons ha^{-1}), S6 (8.31 tons ha^{-1}) and S9 (6.24 tons ha^{-1}). S4 (0.84 tons ha^{-1}) and S8 (2.96 tons ha^{-1}) were the two worst performing species and were also the only two species significantly different to S1 and S2.

On a dryland basis, none of the species were able to produce even 50% of the yield generated by S7. Surely, this puts S7 in a class of its own with respect to its ability of exploiting seasonally regressing climatic requirements, which sub-tropical grasses depend on for productivity (Sinclair et al. 2001).

Table 4.1: Maximum fresh matter yield (tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

	Species									
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1})	SEM
1	38.01 **bcdefghijk**	60.87 **abcdef**	4.30 **k**	21.55 **cdeghijk**	51.26 **abcdefghij**	30.53 **bcdefghijk**	48.57 **bcdefghij**	20.12 **deghijk**	34.40	3.24
2	92.13 **a**	63.15 **abcde**	3.97 **k**	29.57 **bcdefghijk**	30.07 **bcdefghijk**	60.49 **abcdefg**	58.70 **abcdefgh**	20.13 **dfgijk**	44.78	3.26
3	63.90 **abc**	68.74 **ab**	9.33 **jk**	28.36 **cdefghijk**	57.72 **abcdefghi**	68.73 **ab**	63.17 **abcd**	19.84 **efghijk**	47.47	3.24
Average (tons ha^{-1})	64.68	64.25	5.87	26.49	46.35	53.25	56.81	20.03	**42.22**	
SEM	3.24	3.24	3.24	3.24	3.24	3.39	3.24	3.24		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

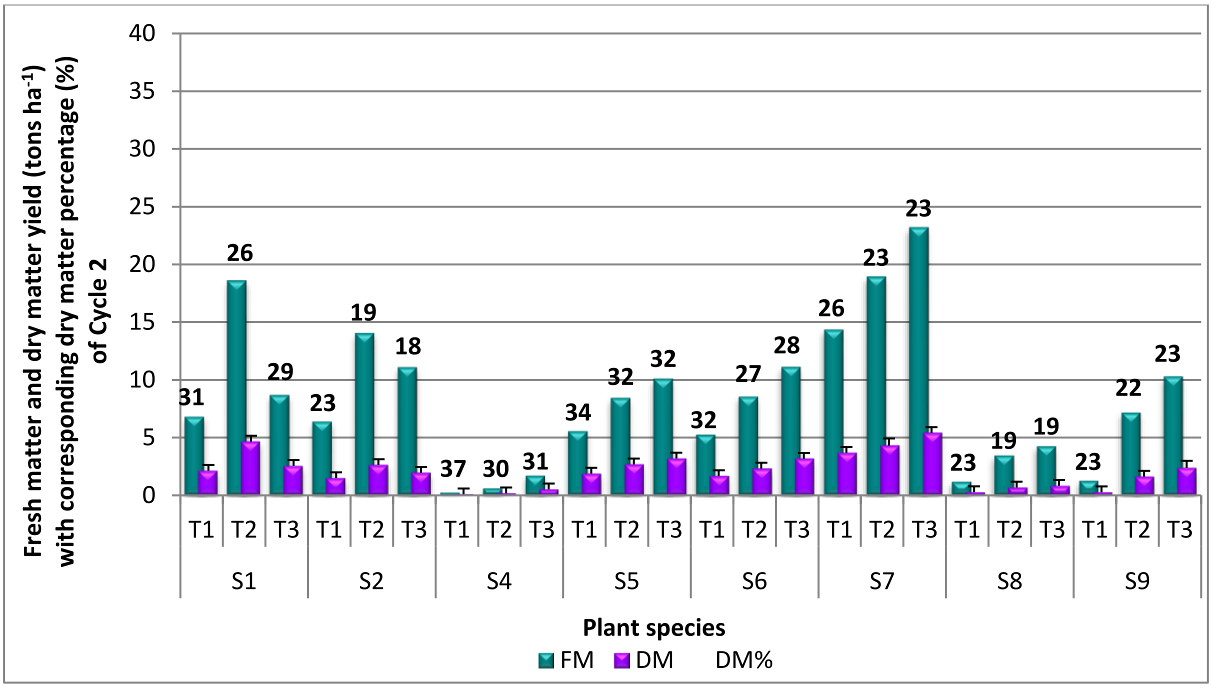


Figure 4.3: Fresh matter (FM) and dry matter (DM) yield with corresponding dry matter percentage (above FM bars – DM %) of eight potential bioenergy sub-tropical *Poaceae* species (S) under three different water treatments (T) from 1 Mar 2015 – 31 May 2015 (Cycle 2). Species: S1 (Guinea grass)*,* S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch)*,* S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

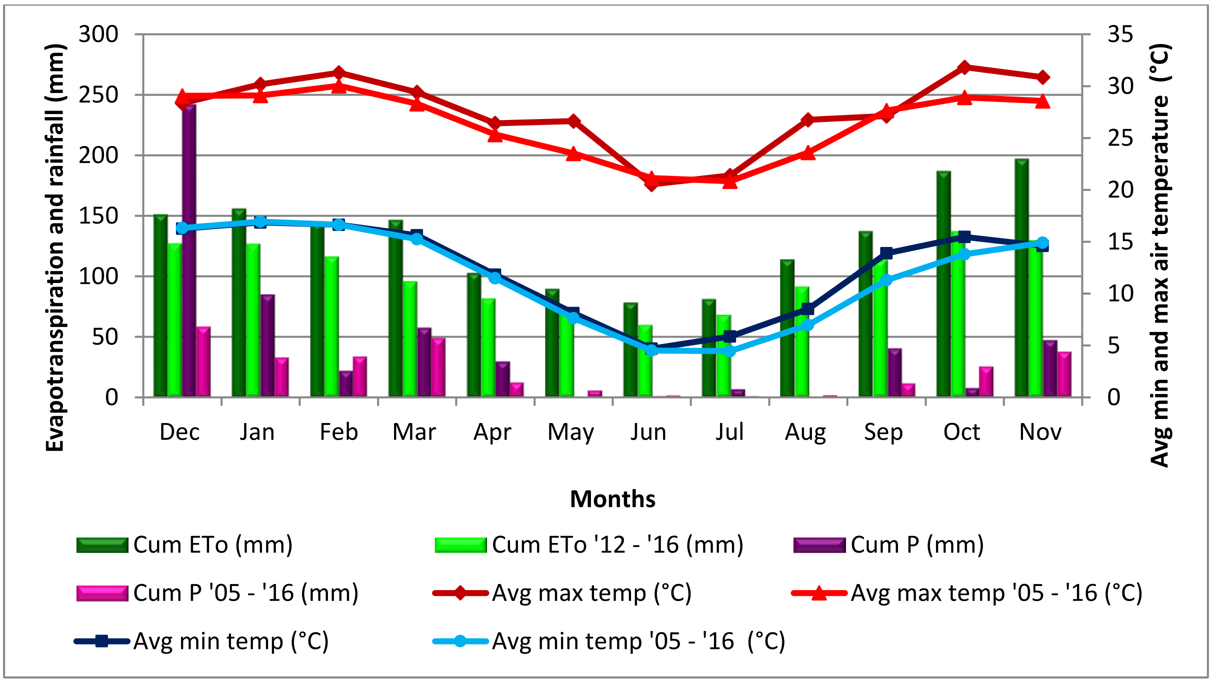


Figure 4.4: Monthly total evapotranspiration and rainfall, as well as minimum and maximum air temperatures measured from the beginning of the trial, from 1 Dec 2014 until the end thereof on 30 Nov 2015, in addition to long-term values for each parameter. [Cum ETo – Cumulative evapotranspiration, Cum P – Cumulative precipitation, Avg max temp – Average maximum temperature, Avg min temp – Average minimum temperature.

4.1.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

The final cycle is the only one of all three cycles to exhibit significant differences across water treatments, species and interactions (Table 4.3). In cycle 3 all treatment combinations of S1, S2, S5, S6 and S8 followed the expected trend of increasing yield according to increasing water application. This may be due to root growth and development which occurred within the different water treatments throughout the successive cycles. The highest yield for treatment combinations of S4 and S7 was produced at T2, followed by T3 and T1. Only S9 exhibited treatment combinations with the highest yield at T3, followed by T1, and the lowest yield at T2 (Figure 4.5).

Only treatment combinations concerning S4 (T1 – 3.48, T2 – 16.36 & T3 – 8.84 tons ha^{-1}) and S9 (T1 – 9.29, T2 – 6.50 & T3 – 22.48 tons ha^{-1}) produced yields lower than the cycle average, (23.01 tons ha^{-1}). At least one treatment combination of all remaining species produced a yield below the cycle average, which were as follows; S1 (T1 – 10.08 & T2 – 19.53 tons ha^{-1}), S2 (T1 – 18.43 tons ha^{-1}), S5 (T1 – 16.72 tons ha^{-1}), S6 (T1 – 17.92 tons ha^{-1}), S7 (T1 – 9.15 & T3 – 18.18 tons ha^{-1}) and S8 (T1 – 11.69 & T2 – 12.88 tons ha^{-1}). All of the treatment combinations mentioned in this paragraph were significantly similar to each other (Table 4.3).

The highest yield was produced by the treatment combination of S2 at T3 (53.83 tons ha^{-1}), with certain treatment combinations of S2 (T2 - 42.31 tons ha^{-1}), S5 (T2 - 46.88 & T3 - 50.13 tons ha^{-1}), S6 (T3 - 32.86 tons ha^{-1}), S7 (T2 - 28.10 tons ha^{-1}) and S8 (T3 - 37.77 tons ha^{-1}) producing significantly similar yields (Table 4.3).

Overall, cycle 3 produced yields which were greater than those of cycle 2, but lower than those of cycle 1. The only exceptions to this trend was the greater values of certain treatment combinations of S5 (T2 – 46.88 & T3 – 50.13 tons ha^{-1}) and S9 (T3 – 22.48 tons ha^{-1}) in cycle 3 as compared to those of cycle 1; S5 (T2 – 29.57 & T3 – 28.36 tons ha^{-1}) and S9 (T3 – 19.84 tons ha^{-1}). In cycle 2 there was only a single treatment combination (S7 T2 – 18.80 tons ha^{-1}) which was greater than that produced in cycle 3 (S7 T2 – 18.48 tons ha^{-1}).

As previously stated, the unexpected results produced by treatment combinations of S4 will be discussed in more detail in section 4.6.

A possible theory regarding the similar trend exhibited by treatment combinations of S7 as compared to those of S4 suggests that this species was perhaps simply cut too short during harvesting and as a result, re-growth was negatively affected and so also biomass production. The low yields of cycle 2, as well as the tufted growth habit of S7 meant that harvesting in cycle 2 was easier and less destructive, with respect to the sample, if harvested with sheers instead of a brush cutter. When workers were able to harvest by hand they cut below 5 cm, meaning to remove as much biomass as possible. Several plots were harvested in this manner before the fault was realised and then rectified for the remaining plots. It is very likely that for S7 the harvest procedure happened to be the most “damaging” at T3, as a result of the trial layout and the harvesting pattern followed during that particular harvest.

Table 4.2: Maximum fresh matter yield (tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Overall average (tons ha^{-1})
FMY (kg)	11.36 **b**	10.51 **b**	0.843 **d**	8.04 **bc**	8.31 **bc**	18.80 **a**	2.96 **cd**	6.24 **bcd**	8.38
SEM	1.34	1.34	1.34	1.34	1.34	1.44	1.34	1.44	
LSD	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	

Water treatments				
	1	2	3	Average (tons ha^{-1})
FMY (kg)	5.12 **b**	9.97 **a**	10.05 **a**	8.38
SEM	0.82	0.84	0.84	
LSD	2.36	2.36	2.36	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Fresh matter yield (FMY), standard error of means (SEM), least significant difference (LSD).

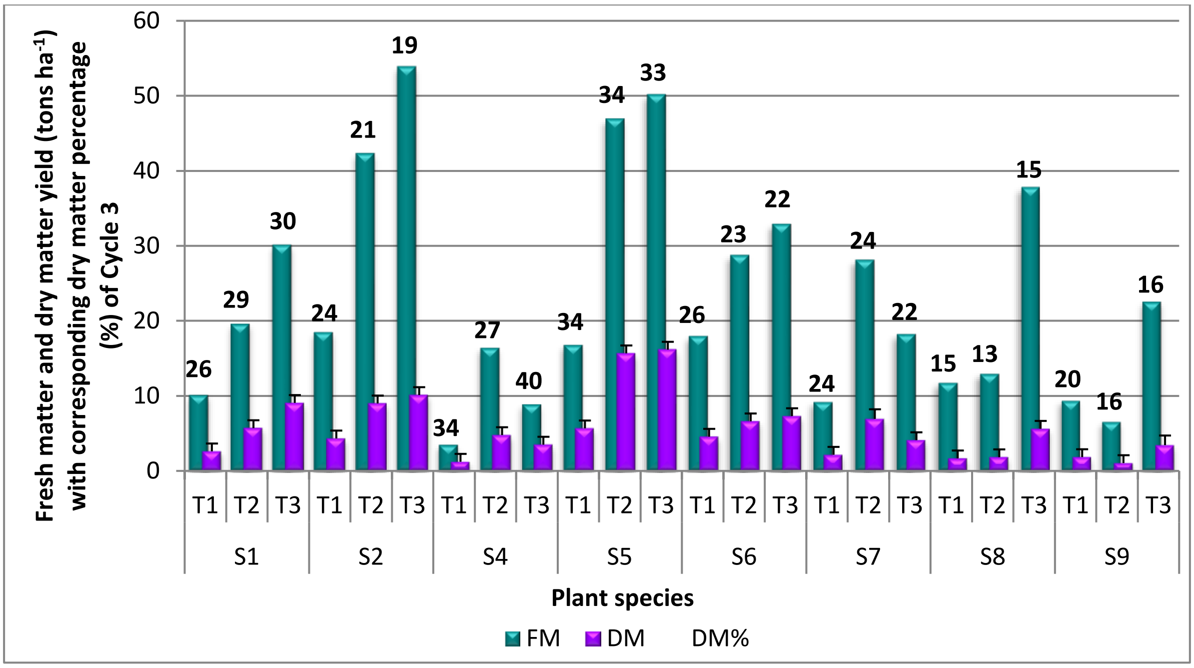


Figure 4.5: Fresh matter (FM) and dry matter (DM) yield with corresponding dry matter percentage (above FM bars – DM %) of eight potential bioenergy sub-tropical *Poaceae* species (S) under three different water treatments (T) from 1 Sep 2015 – 30 Nov 2015 (Cycle 3). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

There is a strong likelihood that re-growth of this species was negatively affected by this harvesting procedure. It is expected that under improved harvest procedures greater yields will be produced by this species, especially at T3. As stated by McNaughton (1979), very few species exhibit the ability to remain productive when subjected to intensive clipping at a height of 4 cm. Youngner (1972) also agrees that dry matter production of grasses decreases with close clipping.

It is strongly suspected that S1 also underwent the same harvesting “fault”, also attributed to the low yields of cycle 2 and the tufted habit of this species. This might offer an explanation for the relatively much lower yields exhibited by treatment combinations of S1 at cycle 3 as compared to cycle 1 and cycle 2. However, it is not possible to compare these findings with available literature since literature represents S1 and S7 either solely or chiefly as a forage species. It is therefore very difficult to determine whether or not the yields attained by treatment combinations of S1 and S7, more particularly in the final cycle, were affected by the harvesting procedure.

Alternatively, the relatively much lower yields of S1 and S7 treatment combinations for cycle 3 as compared to cycle 1 might suggest that both these species are perhaps weak perennials (Dr Wayne Truter personal communication 2017). From a financial point of view, continual expenditure in order to re-establish these crops would very negatively affect profitability, as well as sustainability. The viability of both these species would need to be tested further by perhaps extending the present trial period by at least another year in order to analyse the longevity of these grasses specifically. However, if the outcome confirms that these species are indeed stronger annuals than perennials, then this need not necessarily be a negative result. As stated in the literature review, proficient annual bioenergy species will be needed as rotation crops for food production because annual species offer greater plasticity with respect to market demand and production alterations (Rooney et al. 2007).

Climatic conditions of cycle 3 were generally much harsher than the climatic conditions of cycle 1 (Figure 4.4). Yields across all treatment combinations were generally lower in cycle 3 than those exhibited in cycle 1. This, of course, could also have attributed to the relatively much lower yields treatment combinations concerning S1 and S7 in cycle 3.

In cycle 3 only treatment combinations of S5 (T2 – 46.88 & T3 – 50.13 tons ha^{-1}) yielded compelling increases in production as compared to cycle 1 (T2 – 29.57 & T3 – 28.36 tons ha^{-1}) (Figures 4.2, 4.3 & 4.5). The results attained in a water deficit trial done by Zhou and Yu (2010), suggest that S5 is able to recover fully or at least partially from moderate water stress. However, according Truong and Loch (2004), S5 is able to withstand prolonged drought conditions, and thus they tend to support the notion that this species might be a viable candidate for production on marginal lands.

An outcome which was clearly noted with respect to S5 was that this species was rather slow to establish and thus would require more time to do so as compared to the other species. This may in part be attributed to the enormous root system of this species (Pinners 2014), which naturally would require a large fraction of assimilate partitioning to the below ground biomass. The investment of assimilates to the parts of the plant which will not be harvested does however clearly pay off since only treatment combinations of S5 were able to produce such considerable yield increases under such harsh conditions as endured in cycle 3. Delayed maximum yield production might be a financial inconvenience, but it seems that low initial yields might be more than made up for in years following establishment. According to Truong and Loch (2004) S5 stands can remain productive for more than 20 years with fertilizer supplementation, and thus the slight setback of lower initial yield is truly a minor issue in the bigger scheme of things.

From a bioenergy perspective it is not a frequently exercised approach to discuss the annual fresh matter production of a crop since this value alone states little about the potential energy production of a species. Thus, annual biomass yield on a dry matter basis will be discussed in section 4.4.

Table 4.3: Maximum fresh matter yield (tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

	Species									
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1})	SEM
1	10.08 **fghi**	18.43 **efghi**	3.48 **i**	16.72 **efghi**	17.92 **efghi**	9.15 **ghi**	11.69 **fghi**	9.29 **ghi**	12.10	1.52
2	19.53 **defghi**	42.31 **abcd**	16.36 **efghi**	46.88 **abc**	28.71 **bcdefgh**	28.10 **abcdefghi**	12.88 **fghi**	6.50 **hi**	25.16	1.56
3	30.07 **bcdefg**	53.83 **a**	8.84 **ghi**	50.13 **ab**	32.86 **abcdef**	18.18 **efghi**	37.77 **abcde**	22.48 **cdefghi**	31.77	1.56
Average (tons ha^{-1})	19.89	38.19	9.56	37.91	26.49	18.48	20.78	12.76	**23.01**	
SEM	2.48	2.48	2.48	2.48	2.48	2.67	2.48	2.68		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

4.2 Leaf area index

4.2.1 – Production Cycle 1: Dec 2014 – Feb 2015

The leaf area index (LAI) graphs (Figure 4.6 – 4.8) illustrate the growth curves of each species in each cycle in response to different water treatments by plotting LAI against successive monthly harvest intervals. This data might help to suggest alterations which could be made to the cycle lengths of each particular species under different water regimes in order to optimise the productivity and efficiency of each species.

It would be expected, to a fair degree, that LAI would be correlated to the fresh matter yields of the selected grass species. When comparing the fresh matter yields in section 4.1 with the LAI data in section 4.2 for each respective cycle, it is clear that a rather strong correlation does indeed exist. In cycle 1 species and interactions proved to be statistically significant. It does not come as a surprise to learn that the greatest LAI in cycle 1 was accredited to the treatment combination of S1 at T2 (6.06 m^2 m^{-2}) (Figure 4.6), the same treatment combination which produced by far the largest fresh matter yield in cycle 1 (Figure 4.2). The LAI measurements for treatment combinations of only S1 (T1 - 2.79, T2 - 6.06 & T3 - 4.27 m^2 m^{-2}), S2 (T1 - 3.22, T2 - 2.76 & T3 - 2.54 m^2 m^{-2}), and S7 (T1 - 2.92, T2 - 5.10 & T3 - 3.15 m^2 m^{-2}) were greater than the average LAI of cycle 1 (2.28 m^2 m^{-2}). However, only the treatment combinations of S7 T2 (5.10 m^2 m^{-2}) and S1 T3 (4.27 m^2 m^{-2}) produced significantly similar values to the greatest LAI measurement of the cycle (S1; T2 - 6.06 m^2 m^{-2}) (Table 4.4).

The lowest maximum LAI values were produced by treatment combinations of S4 (T1 – 0.18, T2 – 0.07 & T3 – 0.52 m^2 m^{-2}), S5 (T1 – 0.85, T2 – 1.29 & T3 – 1.27 m^2 m^{-2}), and S9 (T1 – 1.49, T2 – 1.52 & T3 – 1.51 m^2 m^{-2}), which were also substantially lower than the cycle average (Table 4.4).

Only the treatment combinations of S5 (T1 – 0.85, T2 – 1.27 & T3 – 1.29 m^2 m^{-2}) and S8 (T1 – 2.19, T2 – 2.53 & T3 – 2.83 m^2 m^{-2}) followed the generally expected trend of increased LAI according to increased water application (Table 4.4). Treatment combinations of S1 (T1 – 2.79, T2 – 6.06 & T3 – 4.27 m^2 m^{-2}), S7 (T1 – 2.92, T2 – 5.10 & T3 – 3.15 m^2 m^{-2}) and S9 (T1 – 1.49, T2 – 1.52 & T3 – 1.51 m^2 m^{-2}) produced their greatest LAI values at T2, followed by T3 and T1. The treatment combinations of S4 (T1 – 0.18, T2 – 0.07 & T3 – 0.52 m^2 m^{-2}) and S6 (T1 – 1.90, T2 – 1.15 & T3 – 2.61 m^2 m^{-2}) produced their highest value at T3, as would be expected, however, the second highest LAI values were produced at T1, followed by T2. Only the treatment combinations of S2 (T1 – 3.22, T2 – 2.76 & T3 – 2.54 m^2 m^{-2}) exhibited values which were the opposite of that which was expected, producing the highest LAI value at T1, followed by T2 and T3 (Table 4.4).

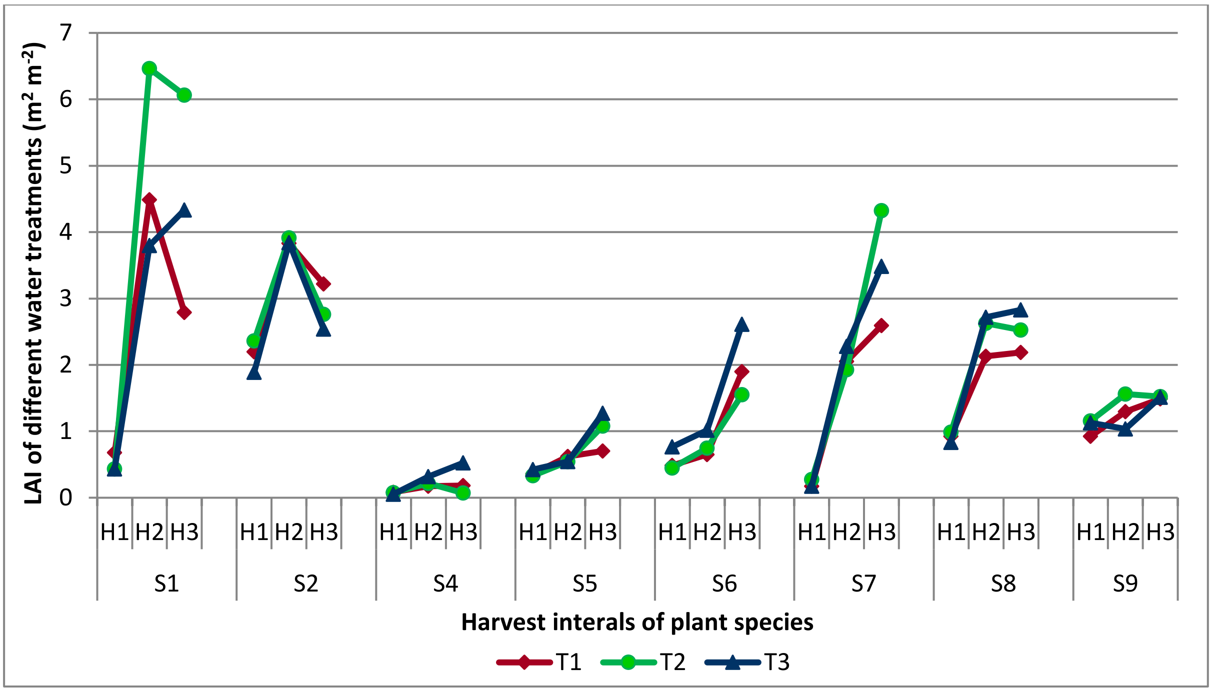


Figure 4.6: Monthly LAI development of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Dec 2014 – 28 Feb 2015 (Cycle 1). Data on statistical significances are presented in Table 4.4. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

When considering treatment combinations across successive harvest intervals then again only S5 and S8 followed the generally expected trend of increasing LAI with increasing water application. The treatment combinations of S5, however, exhibited an exponential increase in LAI across successive harvest intervals, whereas those of S8 experienced a major deceleration in the rate of LAI increase between the second and the third harvest intervals (Figure 4.6). This probably indicates that the treatment combinations of S5 had not achieved maximum productivity by the end of the final harvest and should therefore be tested under cycle lengths greater than three months. However, according to Smeal et al. (2003), a three month growth cycle was the optimal cycle length for maximum dry matter production in Queensland, Australia, under their field trial conditions fairly similar to that of South Africa. Delayed establishment of the species might be a contributing factor to an exponentially increasing LAI. Once individual S5 plants have grown to full maturity, then maximum LAI might very well be attained within three months. A LAI of 14 m^2 m^{-2} after three months of growth was reported by Smeal et al. (2003), compared to a maximum LAI of 1.29 m^2 m^{-2} in the present cycle. Therefore, there might indeed be validity in the theory of delayed establishment, since S5 treatment combinations only managed to produce a fraction of the LAI which might be expected from this species. Another likely contributing factor to the low LAI is the relatively low planting density of 1 m by 0.5 m as compared to a spacing of 0.3 m by 0.3 m used by Pinners (2014). Increasing the planting density is a factor which will most likely lead to increased LAI values, as well as increased dry matter yield.

The treatment combinations of S8 on the other hand exhibited a plateau in LAI production between the second and third month of the growth cycle (Figure 4.6) and thus had most likely achieved maturity by the end of the cycle.

It could therefore be expected that the treatment combinations of S8 would have experience a productivity decline if the harvesting period was extended by another month. A three-month growth cycle for treatment combinations concerning S8 is therefore most likely an ideal growth cycle length, or at least for the seasonal attributes of cycle 1.

The treatment combinations of S6 very closely followed the same trend as those of S5, producing LAI values at an exponentially increasing rate between the first and the final harvest. Besides for the overall greater LAI of S6 treatment combinations as compared to those of S5, the only major difference was that the dry land treatment combination of S6 produced a greater LAI than at T2. It is strongly suspected that the same reason behind the declined fresh matter yield of S6 at T2 (a majority of weaker or smaller plants being planted in T2, Figure 4.2) is responsible for the decline in LAI of this treatment combination. However, the exponential LAI increase across all treatment combinations concerning S6 also suggests that S6 did not reach maximum productivity within three months and thus should also be tested under increased cycle lengths.

The treatment combinations of S1 and S2 varied with respect to those of the other species in that they are the only grasses which exhibited decreased LAI values between the second and third months of cycle 1, with the sole exception of S1 at T3. It is difficult to identify a possible cause behind these results. What seems to be the only logical conclusion is that since these two species produced such large fresh matter yields, (the largests of all species in this cycle), leaf senescence of the lower leaves had taken place between the second and third month of production due to soil nutrient deficiencies or shading.

The treatment combinations of S4, as stated in section 4.1, are not expected to give an accurate representation of the potential of this species. The maximum LAI of 0.52 m^2 m^{-2} attained by S4 at T3 in the present cycle, as compared to the 6 - 12 m^2 m^{-2} reported by Hastings et al. (2009) strongly supports this notion.

4.2.2 – Production Cycle 2: 1 Mar 2015 – 31 May 2015

Only water treatments and species showed statistically significant differences. The mean LAI values of T2 (1.02 m^2 m^{-2}) and T3 (1.11 m^2 m^{-2}) were significantly similar to each other, but significantly greater than that of T1 (0.60 m^2 m^{-2}) (Table 4.5).

The comparably high fresh matter yield of S7 in cycle 2 (Figure 4.3) resulted in S7 also producing the greatest mean LAI of all species (2.89 m^2 m^{-2}) (Table 4.5). The second and third greatest mean LAI values were produced by S1 (1.32 m^2 m^{-2}) and S2 (1.09 m^2 m^{-2}). However, even though both were statistically similar to each other, both were significantly lower than S7. The three species mentioned above were also the only species to produce LAI values in excess of the average across all species, (0.91 m^2 m^{-2}). S6 (0.76 m^2 m^{-2}) could be considered to have produced an intermediate LAI since it was statistically similar to all species besides the greatest and lowest LAI values. S4 (0.08 m^2 m^{-2}) produced the lowest LAI, however, it was not significantly different to any of the next three lowest LAI values, namely; S8 (0.25 m^2 m^{-2}), S5 (0.37 m^2 m^{-2}) and S9 (0.52 m^2 m^{-2}).

Table 4.4: Maximum leaf area index (LAI) values (m^2 m^{-2}) achieved by eight *Poaceae* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

	Species									
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (m^2 m^{-2})	SEM
1	2.79 **bcde**	3.22 **bcd**	0.18 **fg**	0.85 **defg**	1.90 **cdefg**	2.92 **bcde**	2.19 **cdefg**	1.49 **defg**	1.94	0.10
2	6.06 **a**	2.76 **bcde**	0.07 **g**	1.27 **defg**	1.15 **defg**	5.10 **ab**	2.53 **bcdefg**	1.52 **defg**	2.56	0.11
3	4.27 **abc**	2.54 **bcdefg**	0.52 **efg**	1.29 **defg**	2.61 **bcdef**	3.15 **bcd**	2.83 **bcde**	1.51 **defg**	2.34	0.10
Average (m^2 m^{-2})	4.37	2.84	0.26	1.14	1.89	3.73	2.51	1.51	**2.28**	
SEM	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.17		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

The general trend exhibited was that species did not seem to have developed maximum canopies yet by the final harvest of cycle 2 (Figure 4.7). In fact, besides for both S8 and S9, most LAI values generally still seemed to be increasing exponentially by the end of harvest 3.

With the exception of S7, LAI values were also generally much lower in cycle 2 (Figure 4.7) as compared to cycle 1 (Figure 4.6). This data might agree with the statement made in section 4.1.2, suggesting that S7 is perhaps more capable of exploiting regressing climatic conditions necessary for C4 subtropical grass growth.

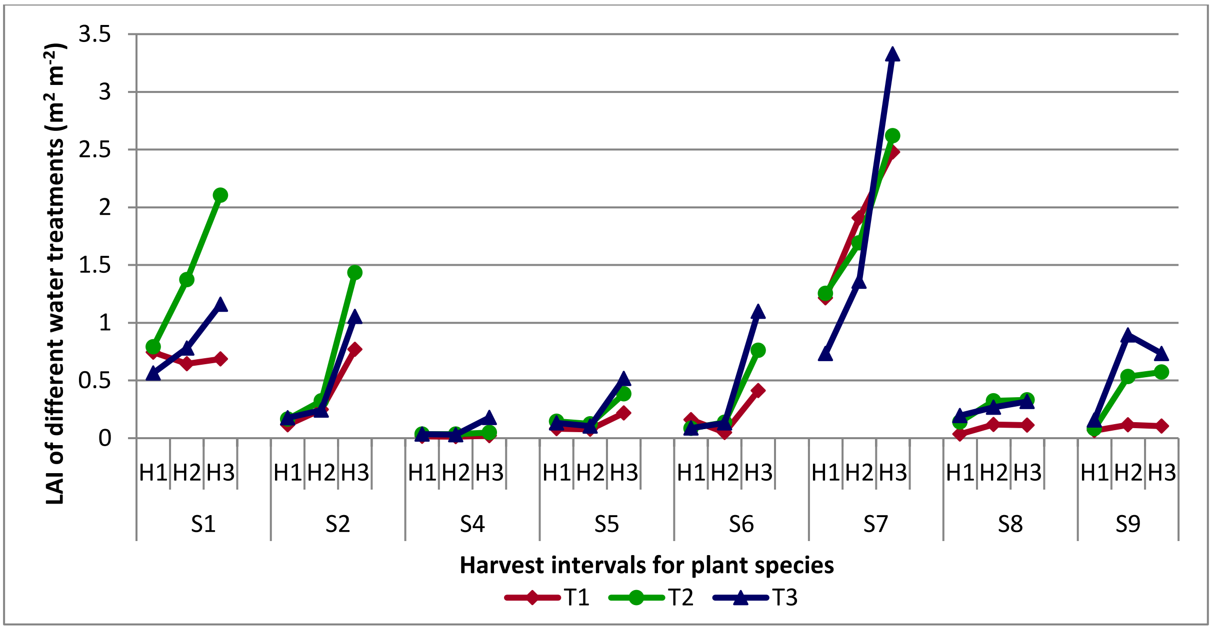


Figure 4.7: Monthly LAI development of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Mar 2015 – 31 May 2015 (Cycle 2). Data on statistical significances are presented in Table 4.5. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.2.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

All main effects were statistically significant; water treatments, species and interactions. Cycle 3 is the only cycle in which the LAI values of all three water treatments were significantly different from each other. T3 was greater than T2, and T2 was greater than T1.

Only the treatment combinations of S4 (T1 – 0.13, T2 – 0.35 & T3 – 0.24 m^2 m^{-2}) and S6 (T1 - 0.31, T2 – 0.33 & T3 – 0.28 m^2 m^{-2}) were lower than the average LAI of cycle 2 (1.61 m^2 m^{-2}), and so across all treatment combinations (Table 4.5).

The treatment combination of S8 at T3 (5.58 m^2 m^{-2}) produced a LAI almost double that of the two next best values; S9 at T3 (3.36 m^2 m^{-2}) and S7 at T2 (2.97 m^2 m^{-2}) (Figure 4.8). The comparably much greater LAI of S8 at T3 versus all other values resulted in this treatment combination being significantly different from all others across cycle 3 (Table 4.6).

Table 4.5: Maximum leaf area index (LAI) values (m^2 m^{-2}) achieved by eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (m^2 m^{-2})
LAI	1.32 **b**	1.09 **bc**	0.08 **e**	0.37 **de**	0.76 **bcd**	2.89 **a**	0.25 **de**	0.52 **cde**	0.91
SEM	0.15	0.15	0.15	0.15	0.15	0.17	0.15	0.17	
LSD	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	

Water treatments				
	1	2	3	Average (m^2 m^{-2})
LAI	0.60 **b**	1.02 **a**	1.11 **a**	0.91
SEM	0.09	0.10	0.10	
LSD	0.27	0.27	0.27	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Leaf area index (LAI), standard error of means (SEM), least significant difference (LSD).

A rather strange outcome with respect to the treatment combination of S8 at T3 in cycle 3 is the alomst doubled LAI as compared to the same treatment combination in cycle 1. However, in cycle 1 S8 at T3 had most definitely approached a maximum LAI, as was clearly seen in the drastic decline in rate of LAI increase from harvest 2 to harvest 3 (Figure 4.6). It is therefore unclear why a substantially larger LAI was produced in the final cycle (especially since the fresh matter yield of S8 at T3 in cycle 3 is only about 60% of that attained in cycle 1), and so without a clear indication of regressing LAI.

The following treatment combinations were significantly similar to the second greatest LAI, produced by S9 at T3, namely; S1 at T3 (2.11 m^2 m^{-2}), S2 at T2 and T3 (T2 - 2.46 & T3 - 2.86 m^2 m^{-2}), S5 at T2 and T3 (T2 - 2.02 & T3 - 1.57 m^2 m^{-2}), S7 at T2 and T3 (T2 - 2.97 & T3 - 2.61 m^2 m^{-2}), S8 at T1, T2 and T3 (T1 - 1.99, T2 - 2.07 & T3 - 1.99 m^2 m^{-2}) and S9 at T1 (1.56 m^2 m^{-2}).

The LAI values across successive harvest intervals for treatment combinations of S4, S5 and S6 were generally at their greatest in the second harvest interval, whereafter they declined in the final harvest interval (Figure 4.8).These decreases however are not expected to be as a result of the treatment combinations reaching maturity, but rather perhaps wilting and thus also shrivelling of leaves in extremely hot and dry conditions (Figure 4.4). The low LAI values of S5 treatment combinations in cycle 3, could once again not compare to the LAI of 14 m^2 m^{-2} reported in literature (Smeal et al. 2003). Therefore, an extension of the present trial is once again recommended in order to determine the potential S5 treatment combinations once established.

The majority of the remaining treatment combinations exhibited a general increase in LAI values across successive harvest intervals, indicating a general lack in maturity attained by the end of the final harvest interval. Once again, these result might in large be attributed to the harsh climatic conditions of cycle 3, which read as follows; average maximum air temperature of 29.9 ^{o}C, average minimum air temperature of 14.7 ^{o}C, cumulative evapotranspiration of 384 mm and cumulative precipitation of 55.3 mm (adapted from Figure 4.4). The average long-term climatic conditions of the Hatfield Experimental Farm between 2005 and 2016 (average maximum air temperature – 28.4 ^{o}C, average minimum air temperature – 13.3 ^{o}C, cumulative evapotranspiration – 126.9 mm and cumulative precipitation – 24.9 mm) are clearly far less harsh than that of cycle 3.

4.3 – Dry matter percentage

From a bioenergy perspective, dry matter percentage (DM %) is one of the parameters which determines the “value” of fresh matter by indicating the utilizable fraction of biomass. A high fresh matter yield may be very deceiving in how it portrays the production potential of a species, as does a low fresh matter yield. Coupling a high fresh matter yield with a low DM %, and vice versa, could very easily result in two species with very different production results producing similar dry matter yields. The analysis of DM % can also help to identify which species still have room for improvement from a biomass “quality” perspective and thus could potentially undergo selective breeding to improve this trait.

Table 4.6: Maximum leaf area index (LAI) values ($m^2 m^{-2}$) achieved by eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average ($m^2 m^{-2}$)	SEM
1	0.70 **fghi**	1.17 **cefghi**	0.13 **i**	0.39 **ghi**	0.31 **i**	1.09 **efghi**	1.99 **bcdefgh**	1.56 **bcdefghi**	0.92	0.108
2	1.34 **cdefghi**	2.46 **bcde**	0.35 **hi**	2.02 **bcdefg**	0.33 **i**	2.97 **bc**	2.07 **bcdef**	1.14 **cefghi**	1.59	0.111
3	2.11 **bcdef**	2.86 **bcd**	0.24 **i**	1.57 **bcdefghi**	0.28 **i**	2.61 **bcde**	5.58 **a**	3.36 **b**	2.33	0.111
Average ($m^2 m^{-2}$)	1.38	2.16	0.24	1.33	0.30	2.23	3.21	2.02	**1.61**	
SEM	0.18	0.18	0.18	0.18	0.18	0.19	0.18	0.19		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

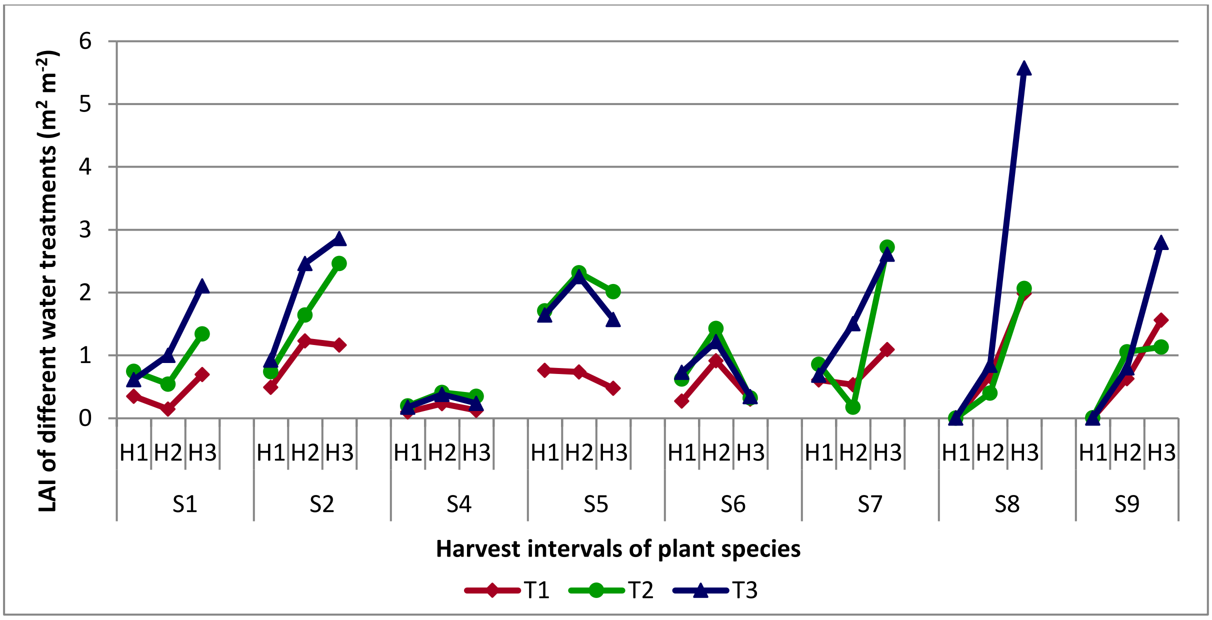


Figure 4.8: Monthly LAI development of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Sep 2015 – 30 Nov 2015 (Cycle 3). Data on statistical significance are presented in Table 4.6. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.3.1 Production Cycle 1: Dec 2014 – Feb 2015

Only species and water treatments produced significantly meaningful differences. The different water treatments of each species generally produced almost identical results at each harvest interval. Also, the unanimous trend of increasing DM % with each successive harvest interval strongly supports the hypothesis that species should be harvested once at maturity instead of multiple times within the same time frame, or at least for this cycle (Figure 4.9).

S1, S4, S5, S6) and S7 produced their greatest DM % values in the dryland water treatment (Figure 4.9). This characteristic is obviously highly desirable when producing bioenergy grasses without the aid of supplementary irrigation. Characteristics such as this provide a platform which could be tested further and perhaps even exploited in order to gain maximum production efficiency from each individual species under different climatic and environmental conditions.

The greatest mean DM % was produced by S6 (43.8 %), and was closely followed by S4 (40.4 %) (Table 4.7). Both species were significantly different to each other and also significantly different to all remaining species. The following species produced the next greatest DM % values and were also significantly similar to each other; S5 (33.4 %), S1 (31.3 %), S9 (30.0 %) and S7 (30.74 %). Besides for the two best performing species, only S5 was able to produce a mean DM % value greater than the average across all species (32.5 %). The two worst performing species were S2 (27.6 %) and S8 (21.9 %), both of which were significantly different to each other; however, S2 was significantly the same as S7 (Table 4.7).

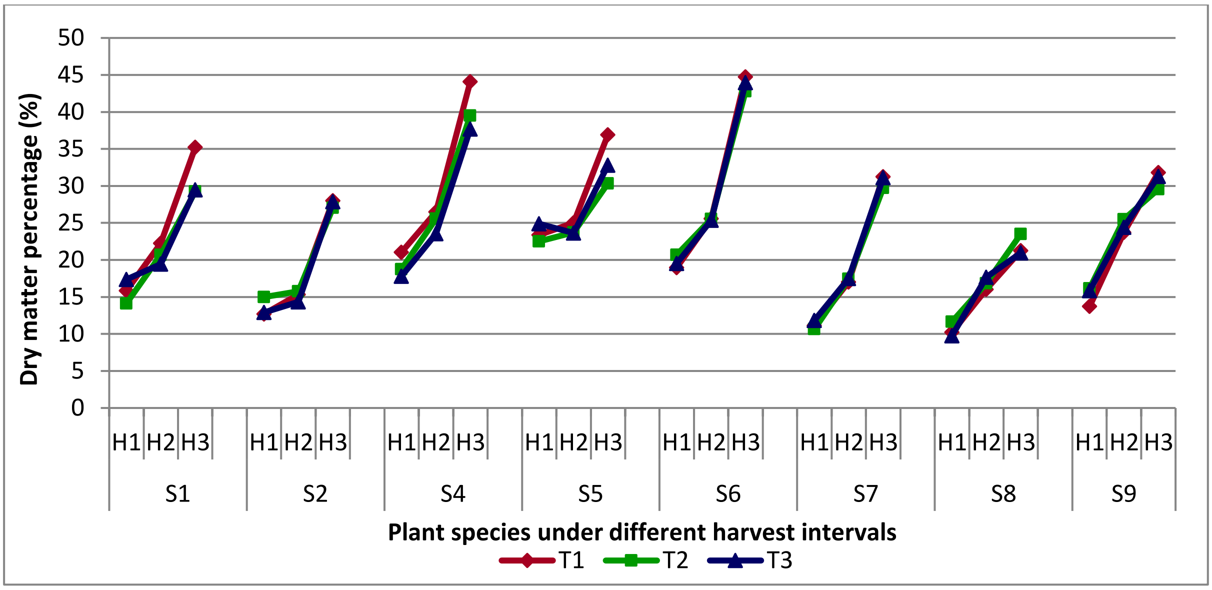


Figure 4.9: Monthly DM % development of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Dec 2014 – 28 Feb 2015 (Cycle 1). Data on statistical significances are presented in Table 4.7. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.3.2 – Production Cycle 2: 1 Mar 2015 – 31 May 2015

Water treatments as well as species exhibited significant differences (Table 4.8). As expected, the dryland water treatment (T1) produced the greatest mean DM % (T1 - 28.6 %), followed by T2 (24.8 %) and T3 (25.5 %); with T2 and T3 being significantly similar to each other, but different from T1. Half of the species were unable to produce a mean DM % value greater than the average across all species (26.3 %), namely; S2 (20.0 %), S8 (20.5 %), S9 (22.8 %) and S7 (24.0 %).

S4 (32.8 %) performed the best out of all species, trailed by S5 with a significantly similar DM % of 32.3 %. The remaining two species, S1 and S6, produced identical DM % values (S1 & S6; 29.1 %) and were significantly different to all other species (Table 4.8).

An extremely interesting result observed in Figure 4.10 is the overall and generally rather severe decline in DM % from the second to the third harvest. Such a unanimous outcome could most likely only be attributed to the physiology of sub-tropical grass species. Assimilate metabolism and relocation to plant roots is commonly observed in such species, thus leading to a decrease in DM % of above ground plant parts.

From a production point of view, there would be strong reasoning to shorten the growth period of cycle 2 and have the final harvest after the second month instead of the third. However, DM % is not a parameter which can be used in isolation to draw a conclusion from with regards to bioenergy potential. These findings will thus be discussed further in section 4.4.

Table 4.7: Maximum dry matter percentage (DM %) values achieved by eight *Poacea* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (%)
DM %	31.3 **c**	27.6 **d**	40.4 **b**	33.4 **c**	43.8 **a**	30.7 **cd**	22.0 **e**	30.9 **c**	32.5
LSD	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	
SEM	0.67	0.67	0.67	0.67	0.67	0.72	0.67	0.67	

Water treatments				
	1	2	3	Average (%)
DM %	34.2 **a**	31.5 **b**	31.9 **b**	32.5
SEM	0.36	0.37	0.36	
LSD	1.03	1.03	1.03	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Dry matter percentage (DM%), standard error of means (SEM), least significant difference (LSD).

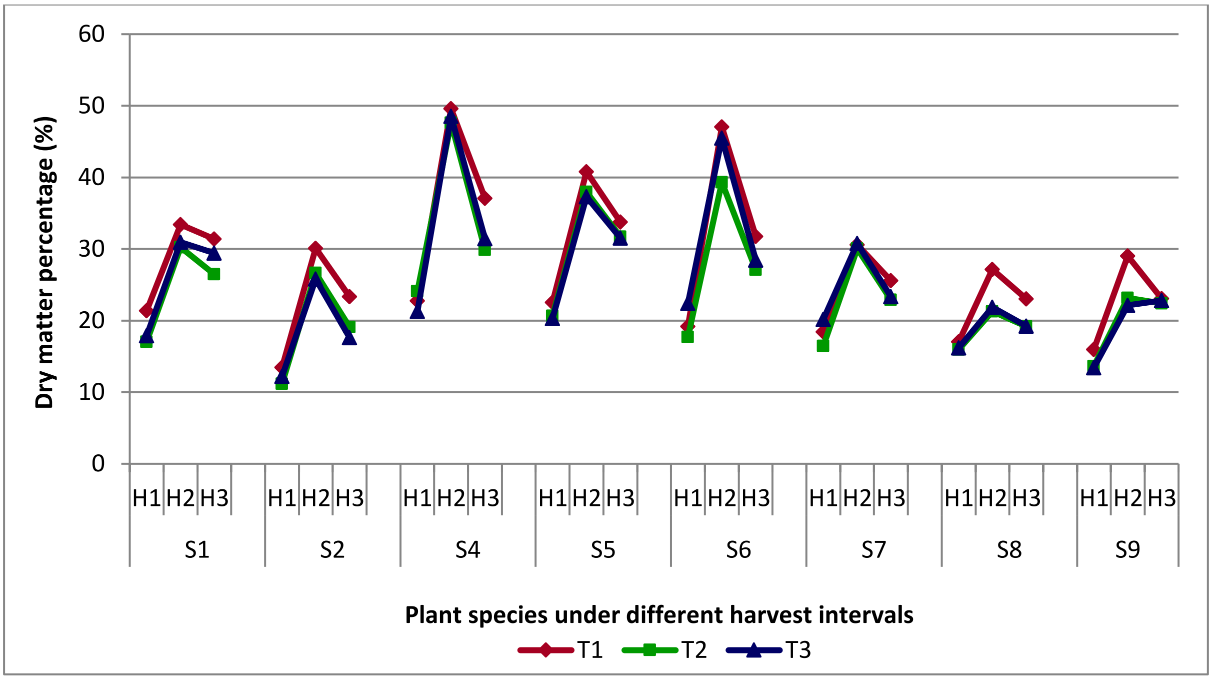


Figure 4.10: Monthly LAI development of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Mar 2015 – 31 May 2015 (Cycle 2). Data on statistical significance are presented in Table 4.8. Species: S1 (Guinea grass)*,* S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch)*,* S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.3.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

Significant differences within species and interactions occurred. The overall average for cycle 3 was 24.4 %, the lowest average of all three cycles. The two greatest DM % values were produced by treatment combinations of S4 (T3 – 39.9 & T1 – 34.3 %) (Table 4.9). Only the treatment combinations of S5 (T1 – 34.3, T2 – 33.6 & T3 – 32.7 %) were significantly similar to S4 at T3. Only the treatment combinations of S1 (T1 – 25.6, T2 – 29.0 & T3 – 30.3 %) produced values which were all also above the cycle average.

All treatment combinations of S2 (T1 – 23.7, T2 – 21.5 & T3 – 18.9 %), S8 (T1 – 14.9, T2 – 12.9 & T3 – 14.8 %) and S9 (T1 – 19.5, T2 – 15.8 & T3 – 15.8 %) produced values below the cycle average.

The treatment combinations of S2, S4, S5 and S9 exhibited a strong trend of increasing DM% with decreasing water application. Only the treatment combinations of S1 strongly opposed this trend, producing increasing DM% values with increasing water application. The treatment combinations for S4, S7 and S8 exhibted ambiguous results with no noticeable trends (Table 4.9). These results, especially when combined with dry matter yield, may offer valuable insight concering species selection for different regions of South Africa.

Table 4.8: Maximum dry matter percentage (DM %) values achieved by eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (%)
DM %	29.1 **b**	20.0 **d**	32.8 **a**	32.3 **a**	29.1 **b**	24.0 **c**	20.5 **d**	22.8 **cd**	26.3
SEM	0.62	0.62	0.62	0.62	0.62	0.67	0.62	0.67	
LSD	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78	

Water treatments				
	1	2	3	Average (%)
DM %	28.6 **a**	24.8 **b**	25.5 **b**	26.3
SEM	0.38	0.39	0.39	
LSD	1.09	1.09	1.09	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Dry matter percentage (DM%), standard error of means (SEM), least significant difference (LSD).

Regarding treatment combinations across successive harvest intervals, the uniformity in trend exhibited in cycle 1 (Figure 4.9) and cycle 2 (Figure 4.10) is virtually non-existent in cycle 3 (Figure 4.11). The results attained in cycle 3 (Figure 4.11) are generally very irregular and inconsistent and it is suspected that these results could perhaps be attributed to the unusually hot and dry weather patterns experienced throughout this cycle (Figure 4.4). Therefore, a repeat of the present trial or at the very least the present cycle, should be strongly considered in order to establish whether or not such ambiguous results are to be expected during this cycle.

With respect to the treatment combinations across successive harvest intervals, a net DM % decline from the two sorghum varieties (Figure 4.11) is a rather unexpected result, especially since literature sets claim to the drought hardiness of this species (Rooney et al. 2007). However, it must be kept in mind that S8 and S9 were replanted and had to grow from seed in every cycle. It is thus suspected that the exposure of seedlings to such harsh climatic conditions (Figure 4.4) may have resulted in a much weaker stand which was not able to maintain or increase initial DM % values.

Only the treatment combinations of S5 generated DM % values which increased with time across each successive harvest interval (Figure 4.11). This is an extremely valuable characteristic with regards to bioenergy crop selection because the treatment combinations of S5 portray a trait of consistency and reliability.

The treatment combinations of S1 and S4 exhibited fairly similar trends to that of S5, with the exception of severe DM % declines at T1 and T2, respectively, between the second and third harvest interval (Figure 4.11). The decline experienced by S1 at T1 is most likely due to the fact that the water stress experienced at T1 was above the tolerance threshold of this treatment combination and thus led to a biomass quality decline. As has already been stated, the treatment combinations of S4 are not expected to give an accurate representation of this species in the present trial and therefore it is not recommended that the data attained on this species be too rigorously analysed.

The majority of remaining treatment combinations for S6, S7, S8 and S9 exhibited extremely strong trends of decreasing DM % values across successive harvest intervals. However, as stated earlier in this section, the ambiguous results attained across treatment combinations of successive harvest intervals make it difficult to draw sound conclusions regarding DM% values and outcomes of cycle 3.

4. 4 - Dry matter biomass yields

Dry matter yield combines the outcome of both fresh matter yield and dry matter percentage. It is for this reason that dry matter yield is such an important parameter to analyse when considering bioenergy crops.

Table 4.9: Maximum dry matter percentage (DM %) achieved by eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

	Species									
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (%)	SEM
1	25.6 **defg**	23.7 **efgh**	34.3 **ab**	34.3 **ab**	26.3 **cdefg**	23.5 **efgh**	14.9 **jkl**	19.5 **ghijkl**	25.3	0.49
2	29.0 **bcdef**	21.5 **fghijk**	27.1 **bcdef**	33.6 **abc**	23.2 **efghi**	24.5 **defgh**	12.9 **l**	15.8 **ijkl**	23.5	0.51
3	30.3 **bcde**	18.9 **ghijkl**	39.9 **a**	32.7 **abcd**	22.2 **fghijk**	22.4 **fghij**	14.8 **kl**	15.8 **hijkl**	24.6	0.51
Average (%)	28.3	21.3	33.8	33.5	23.9	23.5	14.2	17.0	**24.4**	
SEM	0.81	0.81	0.81	0.81	0.81	0.87	0.81	0.87		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

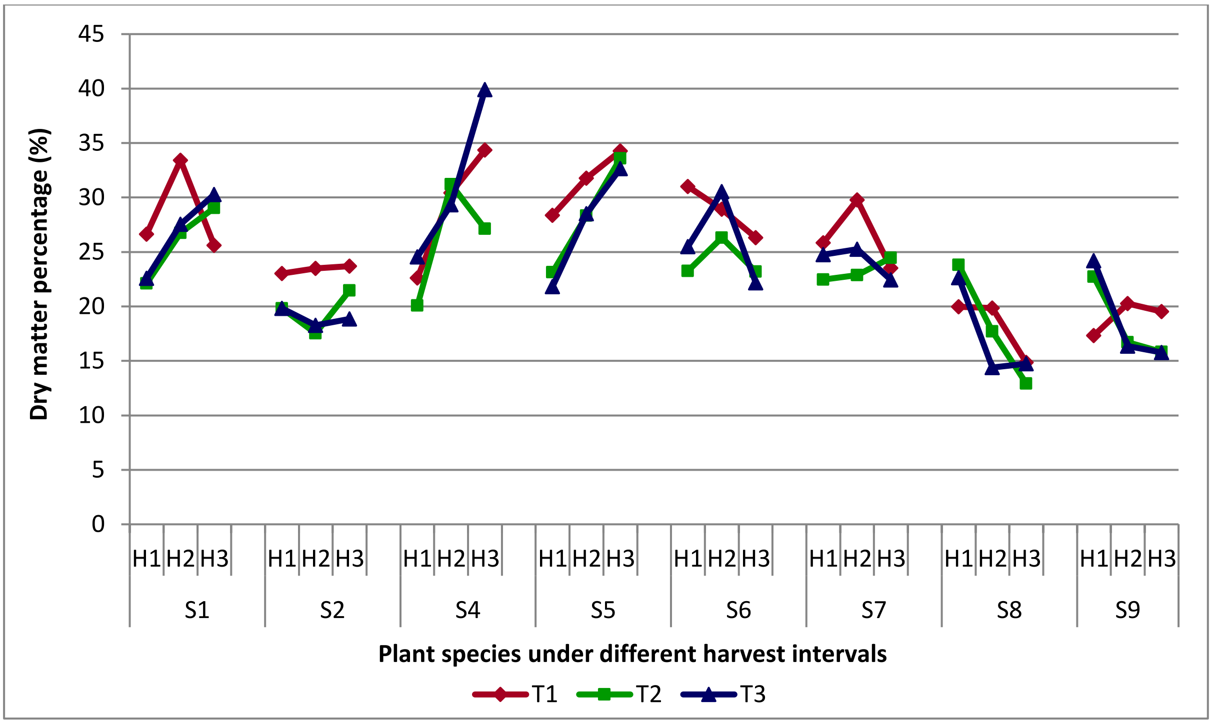


Figure 4.11: Monthly DM % of eight potential bioenergy sub-tropical *Poaceae* species (S) within three harvest (H) intervals under three different water treatments (T) from 1 Sep 2015 – 30 Nov 2015 (Cycle 3). Data on statistical significances are presented in Table 4.9. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.4.1 Production Cycle 1: Dec 2014 – Feb 2015

Species and interaction effect differences were statistically significant, as was also the case for the fresh matter yield of cycle 1.

All treatment combinations of S1 (T1 – 13.29, T2 – 27.01 & T3 – 18.86 tons ha^{-1}) and S2 (T1 – 17.09, T2 – 17.00 & T3 – 19.18 tons ha^{-1}) were significantly similar to the greatest yield of cycle 1 (S1;T2 – 27.01 tons ha^{-1}). Two treatment combinations of S6 (T1 – 23.09, & T3 – 25.33 tons ha^{-1}), S7 (T2 – 18.09 & T3 – 21.31 tons ha^{-1}) and S8 (T2 – 13.86 & T3 – 13.28 tons ha^{-1}) were also significantly similar to the greatest yield of cycle 1. All remaining treatment combinations produced yields lower than the cycle average of 13.00 tons ha^{-1} (Table 4.10).

Only treatment combinations of S5 (T1 – 7.95, T2 – 8.93 & T3 – 9.45 tons ha^{-1}) and S7 (T1 – 9.53, T2 – 18.09 & T3 – 21.31 tons ha^{-1}) followed the expected trend of increased dry matter production with increased water application. Treatment combinations of S2 (T1 – 17.09, T2 – 17.00 & T3 – 19.18 tons ha^{-1}), S4 (T1 – 3.56, T2 – 1.56 & T3 – 1.94 tons ha^{-1}) and S6 (T1 – 23.09, T2 – 12.92 & T3 – 25.33 tons ha^{-1}) produced their greatest yield at T3, but their lowest yield at T2 (Figure 4.2). The fact that the dryland treatment combination of S6 produced a significantly similar yield to the greatest yield of cycle 1 strongly substantiates the theory set forward in section 4.1.1, suggesting that the majority of weaker or smaller plants were planted in T2 plots. It has also been made clear that the treatment combinations of S4 should not be analysed too intensely within this trial. The treatment combinations S2 at

T1 also only produced a slightly greater yield than that of S2 at T2, mostly likely attributed to these stands not having fully established by the end of cycle 1.

The treatment combinations of S1 (T1 – 13.29, T2 – 27.01 & T3 – 18.86 tons ha^{-1}) and S8 (T1 – 10.31, T2 – 13.86 & T3 – 13.28 tons ha^{-1}) produced their greatest yield at T2, followed by T3 and T1 (Figure 4.2). A very large yield attained by replicate two of treatment combination S1 at T2 resulted in the large average yield of this treatment combination, however, it is uncertain what caused this spike in production. The treatment combinations of S8 were mostly likely affected to a fair degree by birds which ate the seeds, thus leading to the generally unexpected results.

Only the treatment combinations of S9 (T1 – 6.42, T2 – 5.95 & T3 – 6.20 tons ha^{-1}) produced the greatest yield at the dryland water treatment, followed by T3 and T2 (Figure 4.2). These values are, however, fairly similar to each other and thus as was the case with fresh matter yield, it is expected that S9 had already reached its optimal water requirement at T1, rendering additional water as surplus.

Previously it was stated that neither fresh matter yield nor dry matter percentage should in isolation be considered as representative parameters in determining the potential of a bioenergy crop. The treatment combinations of S6 and S8 serve as good examples to effectively illustrate the reasoning behind this statement. The treatment combination of S6 at T3 produced the lowest maximum fresh matter yield of the top five treatment combinations in cycle 1, but as a result of a very high dry matter percentage the treatment combinations of S6 at T1 and T3 produced the second and third greatest dry matter yields, only slightly behind that of S1 at T2 (Figure 4.2). The treatment combination of S8 at T3 on the other hand produced a fresh matter yield which was on par with the majority of the top five species. However, S8 treatment combinations generated the lowest dry matter percentage of all treatment combinations in this cycle and thus also a dry matter yield which was considerably lower than the treatment combinations it was on par with from a fresh matter yield perspective (Figure 4.2).

The fact that the dryland treatment combinations of S1, S2 and S6 produced greater yields than the dryland treatment combination of S8 might in actual fact suggest that S8 is not the most water use efficient species of those discussed in the literature review. This notion might carry even more validity, especially since the cumulative rainfall for the duration of cycle 1 was 356 mm (Figure 4.1), very close to the minimal seasonal water requirements of this species, according to Ramos et al. (2012).

Table 4.10: Maximum dry matter yield (DMY, tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1})	SEM
1	13.29 **abcdefghi**	17.09 **abcdefg**	1.94 **fhi**	7.95 **defghi**	23.09 **abc**	9.53 **cdefghi**	10.31 **bcdefghi**	6.42 **defghi**	11.20	1.08
2	27.01 **a**	17.00 **abcdefgh**	1.56 **i**	8.93 **cdefghi**	12.92 **bcdefghi**	18.09 **abcdef**	13.86 **abcdefghi**	5.95 **defghi**	13.17	1.08
3	18.86 **abcde**	19.18 **abcde**	3.56 **fghi**	9.45 **cdefghi**	25.33 **ab**	21.31 **abcd**	13.28 **abcdefghi**	6.20 **efghi**	14.64	1.08
Average (tons ha^{-1})	19.72	17.76	2.35	8.78	20.45	16.31	12.48	6.19	**13.00**	
SEM	1.10	1.10	1.10	1.10	1.10	1.16	1.10	1.10		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

4.4.2 – Production Cycle 2: 1 Mar 2015 – 31 May 2015

Once again, as was the case in the previous cycle, the effects which were significant on a fresh matter yield basis were also significant on a dry matter yield basis. In cycle 2 water treatments and species were significant (Table 4.11).

The mean yields of the two irrigation treatments (T2 – 2.38 & T3 – 2.49 tons ha^{-1}) were once again significantly similar to each other. The dryland water treatment (T1 – 1.43 tons ha^{-1}) produced the lowest mean yield and was also significantly different to the two irrigation treatments (Table 4.11). T1 only managed to produce 60 % and 57 % of the dry matter yield of T2 and T3, respectively. This is important data to keep in mind as it could result in a shortage of feedstock availability throughout the current cycle as well as the three month winter period which follows this cycle. With this being said, it only places more emphasis on how important it is to choose the correct bioenergy species for proficient energy production. The fact that the average yield of cycle 2 (Figure 4.3) was also only 16 % of that produced in cycle 1 (Figure 4.2) also very strongly substantiates this statement.

Species exhibited very similar significance trends across dry matter yields as compared to fresh matter yields for cycle 2. Brazilian grass (S7) again produced the greatest mean yield S7 (4.46 tons ha^{-1}) and was significantly different to all other species (Table 4.11). Guinea grass (S1) produced the second greatest mean yield (3.11 tons ha^{-1}) and was also the only species besides S7 to produce a yield greater than the average across all species (2.10 tons ha^{-1}). Even though S2 (2.02 tons ha^{-1}), S5 (2.58 tons ha^{-1}) and S6 (2.38 tons ha^{-1}) produced dry matter yields relatively much lower than S1, these species were all still significantly similar to S1.

However, all three species were also significantly similar to the much lower yielding species, S9 (1.42 tons ha^{-1}). The remaining two species, S8 (0.59 tons ha^{-1}) and S4 (0.26 tons ha^{-1}), not only produced significantly similar yields, but also considerably lower yields than all other species, although significantly similar to S8 (Table 4.11).

4.4.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

Clearly, there is a very strong correlation between the trends in fresh and dry matter yields, since once again the same effects which were significant for fresh matter yield were also significant for dry matter yield in this cycle; water treatments, species and interactions.

The treatment combination of S5 at T3 produced the greatest dry matter yield of cycle 3, and was also significantly different to all other treatment combinations, with the exception of S5 at T2. Only the treatment combinations of S5 (T1 – 5.67, T2 – 15.67 & T3 – 16.16 tons ha^{-1}) were all greater than the cycle average (5.62 tons ha^{-1}), with all treatment combinations of S4 (T1 – 1.22, T2 – 4.77 & T3 – 3.50 tons ha^{-1}), S8 (T1 – 1.67, T2 – 1.82 & T3 – 3.44 tons ha^{-1}) and S9 (T1 – 1.84, T2 – 1.04 & T3 – 3.44) being below or equal to the cycle average (Table 4.12).

Table 4.11: Maximum dry matter yield (DMY, tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1})
DMY	3.11 **b**	2.02 **bc**	0.26 **d**	2.58 **bc**	2.38 **bc**	4.46 **a**	0.59 **d**	1.42 **cd**	2.10
SEM	0.29	0.29	0.29	0.29	0.29	0.32	0.29	0.32	
LSD	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	

Water treatments				
	1	2	3	Average (tons ha^{-1})
DMY	1.43 **b**	2.38 **a**	2.50 **a**	2.10
SEM	0.18	0.18	0.18	
LSD	0.52	0.52	0.52	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Dry matter percentage (DM%), standard error of means (SEM), least significant difference (LSD).

All treatment combinations of S1 (T1 – 2.61, T2 – 5.71 & T3 – 9.06 tons ha^{-1}), S2 (T1 – 4.33, T2 – 9.00 & T3 – 10.11 tons ha^{-1}), S5 (T1 – 5.67, T2 – 15.67 & T3 – 16.16 tons ha^{-1}), S6 (T1 – 4.56, T2 – 6.61 & T3 – 7.30 tons ha^{-1}) and S8 (T1 – 1.67, T2 – 1.82 & T3 – 3.44 tons ha^{-1}) followed the generally expected trend of increased dry matter yield with increased water application. The treatment combinations of S4 (T1 – 1.22, T2 – 4.77 & T3 – 3.50 tons ha^{-1}) and S7 (T1 – 2.15, T2 – 6.93 & T3 – 4.10 tons ha^{-1}) produced their greatest yields at T2, followed by T3 and T1. The treatment combinations of S9 (T1 – 1.84, T2 – 1.04 & T3 – 3.44 tons ha^{-1}) contrary to S4 and S7, exhibited the lowest yield at T2, followed by T1 and T3 (Table 4.12).

Again, the importance of not relying on fresh matter yield or dry matter percentage in isolation in order to substantiate the bioenergy potential of a treatment combination is clearly illustrated when comparing the fresh matter and dry matter yields of this cycle. On a fresh matter basis the treatment combination S2 at T3 produced the greatest yield, followed by S5 at T3. However, when considering dry matter yield, S5 at T3 produced not only the greatest yield of the cycle, but was also significantly different to that of S2 at T3 (Table 4.12).

4.4.4 Overview of cumulative dry matter yields for cycles 1, 2 and 3

The performance of each treatment combination through successive cycles might reveal insight into the likely performance of each species or treatment combination in the following year. For instance, by examining the trends exhibited by each treatment combination throughout the first year of cultivation, it might be possible to determine which species demonstrate stronger annual traits and which demonstrate stronger perennial traits. Also, with very simple calculations on the dry matter yield of each treatment combination at successive harvest intervals it is possible to crudely estimate whether or not it might make more sense to harvest more frequently than only every three months.

However, before speculations are made with regards to theoretical yields, it would be best to first analyse legitimate values, that is to say, annual dry matter yields.

It is important to determine the dry matter production of each treatment combination on a per annum basis since this will indicate how much feedstock could be produced, and thus, how much energy could be generated per year. With respect to total annual dry matter production, only species and interactions were statistically significant.

Guinea grass (S1) at T2 (37.37 tons ha^{-1}) produced the greatest annual dry matter yield of all treatment combinations, trailed by S6 at T3 (35.79 tons ha^{-1}) and S2 at T3 (31.24 tons ha^{-1}). The latter two treatment combinations, as well as all of the following, were all significantly similar to S1 at T2; S1 at T3 (30.47 tons ha^{-1}), S2 at T1 and T2 (T1 – 22.90 tons ha^{-1} & T2 – 28.62 tons ha^{-1}), S5 at T2 and T3 (T2 – 27.28 tons ha^{-1} & T3 – 28.80 tons ha^{-1}), S6 at T1 (29.31 tons ha^{-1}) and S7 at T2 and T3 (T2 – 28.52 tons ha^{-1} & T3 – 30.81 tons ha^{-1}) (Table 4.13). All the treatment combinations mentioned above produced greater yields than the average annual production (20.61 tons ha^{-1}).

Table 4.12: Maximum dry matter yield (DMY, tons ha^{-1}) achieved by eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1})	SEM
1	2.61 **ef**	4.33 **def**	1.22 **f**	5.67 **cdef**	4.56 **cdef**	2.15 **ef**	1.67 **ef**	1.84 **ef**	3.01	0.37
2	5.71 **cdef**	9.00 **cd**	4.77 **cdef**	15.67 **ab**	6.61 **cdef**	6.93 **cdef**	1.82 **ef**	1.04 **f**	6.44	0.38
3	9.06 **cd**	10.11 **bc**	3.50 **def**	16.16 **a**	7.30 **cde**	4.10 **def**	5.62 **cdef**	3.44 **def**	7.41	0.38
Average (tons ha^{-1})	5.79	7.81	3.16	12.50	6.16	4.39	3.04	2.11	**5.62**	
SEM	0.61	0.61	0.61	0.61	0.61	0.65	0.61	0.65		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

Only the treatment combinations of S2 produce yields significantly similar to the greatest yield across all three water treatments. Since the unforeseen establishment complications experienced by S6 at T2 were self-rectified in cycle 3 it is strongly suspected that in future production cycles all treatment combinations of S6 would also be significantly similar to the greatest yield. These results might suggest that treatment combinations of S6 and S2 are the most versatile with respect to establishment and production in regions with annual rainfall ranging between 580 – 950 mm, as was simulated in the present study (Figure 4.1).

All treatment combinations of S2 (T1 – 22.90, T2 – 28.62 & T3 – 31.24 tons ha^{-1}), S4 (T1 – 3.24, T2 – 6.51 & T3 – 7.57 tons ha^{-1}), S5 (T1 – 15.50, T2 – 27.28 & T3 – 28.80 tons ha^{-1}), S7 (T1 – 15.37, T2 – 28.52 & T3 – 30.81 tons ha^{-1}), S8 (T1 – 12.26, T2 – 16.36 & T3 – 19.72 tons ha^{-1}) and S9 (T1 – 8.53, T2 – 8.60 & T3 – 10.06 tons ha^{-1}) followed the generally expected trend of increased dry matter production with increasing water application. Treatment combinations of S1 (T1 – 18.02, T2 – 37.37 & T3 – 30.47 tons ha^{-1}) produced the greatest yield at T2, followed by T3 and T1, while treatment combinations of S6 (T1 – 29.31, T2 – 21.84 & T3 – 35.79 tons ha^{-1}) produced the lowest yield at T2, followed by T1 and T3 (Table 4.13).

The treatment combinations of S4 and S9 performed the worst on a dry matter yield basis, as would be expected based on results attained throughout all three growth cycles. Thus, the demand for annual biomass species which could be used as rotation crops (Rooney et al. 2007) might not be as successfully fulfilled by treatment combinations of S9 as by S8, according to the results attained within the present trial.

Figure 4.12 illustrates the annual dry matter yield of each treatment combination and the contribution of each cycle to the total dry matter yield. A noticeable improvement regarding the dry matter production of S5 treatment combinations from cycle 1 to cycle 3 is clearly evident in Figure 4.12. Also, the theory concerning S1 and S7 perhaps being weak perennials might also be substantiated in Figure 4.12. Besides the treatment combinations of S9, only those of S1 and S7 produced such relatively low yields in cycle 3 as compared to cycle 2.

Surely such poor yields in a cycle where all other treatment combinations, (except those of S9), managed generally much greater yields as compared to the previous cycle, suggest a decline in the production potential. Once again, perhaps the two most likely reasons for such a production decline might be severe harvesting procedures or poor perennial characteristics of these species.

It was previously stated that it might be possible to crudely determine whether or not greater harvest frequencies would be more proficient than if treatment combinations were harvested only three times per year. Theoretical yields were determined by multiplying the yield of each respective harvest interval by the necessary factor to produce a total of nine production months per year. The theoretical yields for each respective cycle were added to result in a yield which would have been produced over nine months, as was the case with three growth cycles of three months each. The sum of the monthly harvest intervals was thus multiplied by a factor of 3, and the sum of the two-monthly harvest intervals was multiplied by a factor of 1.5. The results for the theoretical dry matter yield of each treatment combination versus the actual final dry matter yield are presented in Figure 4.13.

Table 4.13: Annual maximum dry matter yield (DMY, tons ha^{-1} yr^{-1}) achieved by eight *Poaceae* species exposed to three water treatments from 1 Dec 2014 – 30 Nov 2015 (Annual production).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (tons ha^{-1} yr^{-1})	SEM
1	18.02 **cdefghij**	22.90 **abcdefg**	3.24 **ik**	15.50 **cdefghijk**	29.31 **abc**	15.37 **cdefghijk**	12.26 **defghijk**	8.53 **hijk**	15.64	1.51
2	37.37 **a**	28.62 **abcd**	6.51 **gijk**	27.28 **abcdef**	21.84 **bcdefgh**	28.52 **abcde**	16.36 **cdefghijk**	8.60 **ghijk**	21.89	1.51
3	30.47 **abc**	31.24 **abc**	7.57 **ghijk**	28.80 **abcd**	35.79 **ab**	30.81 **abc**	19.72 **cdefghi**	10.06 **eghijk**	24.31	1.51
Average (tons ha^{-1} yr^{-1})	28.62	27.58	5.77	23.86	28.98	24.90	16.11	9.06	**20.61**	
SEM	1.44	1.44	1.44	1.44	1.44	1.48	1.44	1.44		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

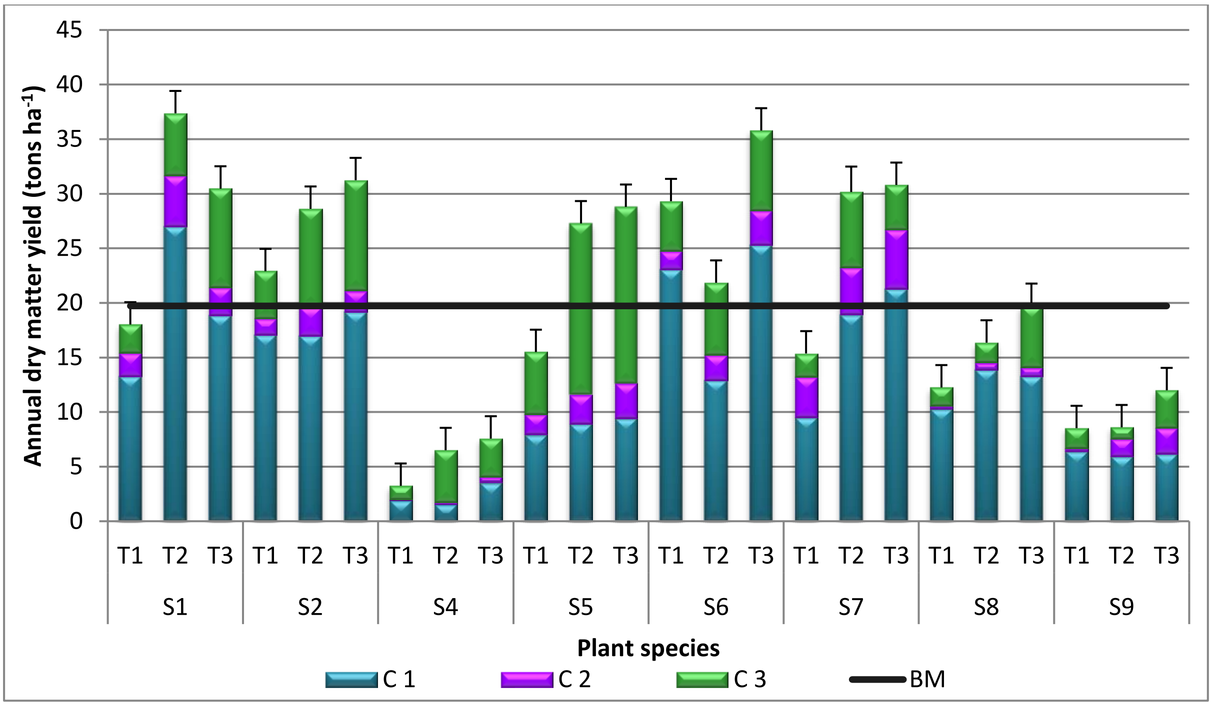


Figure 4.12: Annual final dry matter yield of each species (S) in each water treatment (T) as stacked dry matter yield values for cycle 1 (C1), cycle 2 (C2) and cycle 3 (C3), as compared to the bench mark (BM) - *S. bicolor* (S8). Data on statistical significance are presented in Table 4.13. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Smaller variations between the actual dry matter yield and the two theoretical yields were generally observed at T1, as compared to the two irrigation treatments. The greatest yields of each treatment combination were generally still attained by the actual yields instead of the theoretical yields. However, even where actual yields were not greater than the theoretical yields, the differences were too small to warrant more frequent harvesting and the financial implications thereof.

The only treatment combinations able to produce a fairly greater theoretical yield than an actual yield, and so across all three water treatments, was that of S9. However, unlike perennial species, the theoretical yields of the two sorghum varieties (S8 & S9) would not only require greater input costs with respect to more frequently incurred harvesting costs, but replanting costs as well. It is thus strongly suspected that the greater theoretical yield of S9 treatment combinations would not be great enough to justify the increased financial expenses.

The two irrigation treatments (T2 and T3) generally exhibited much greater actual yields as compared to theoretical yields, with the sole exception of the already addressed S9 treatment combinations. On a financial and production basis it would thus make even less sense to harvest more frequently in areas with greater rainfall as compared to the annual rainfall of Hatfield, Pretoria. These results, though crude, clearly indicate that more frequent harvesting would in actual fact be far less productive while also a great deal more expensive.

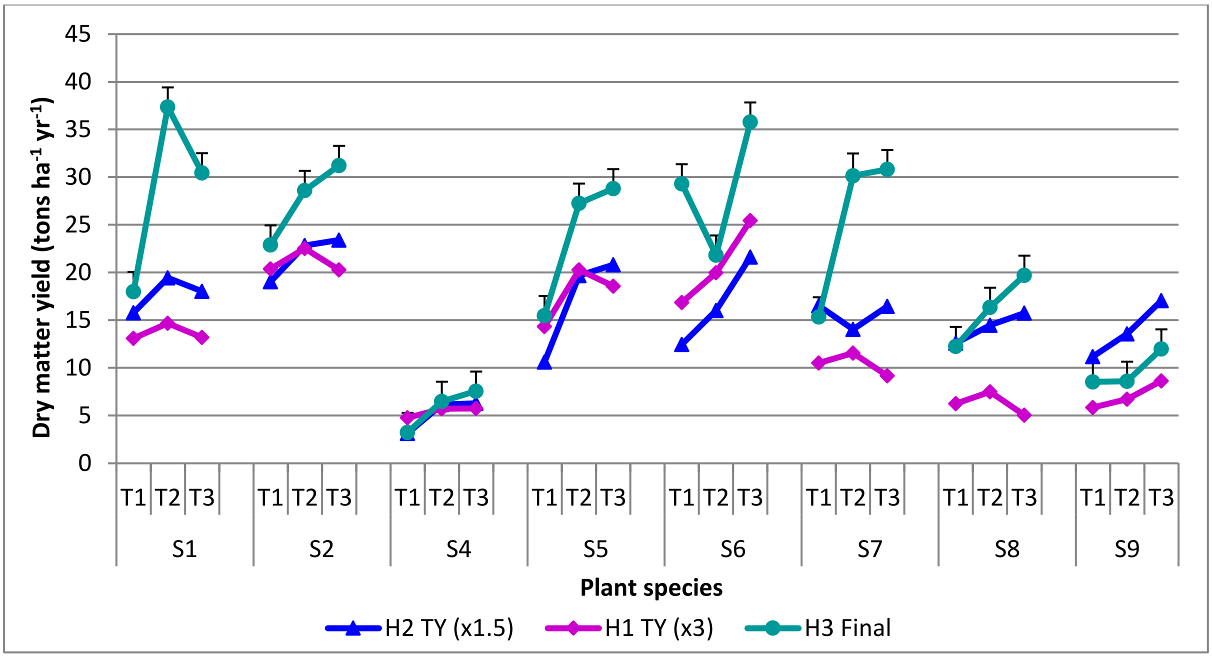


Figure 4.13: Actual annual dry matter yield (H3 Final) and theoretical annual dry matter yield for monthly (H1 TY) and two-monthly (H2 TY) harvest intervals for different species (S) and different water treatments (T) from 1 Dec 2014 - 30 Nov 2015. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Within the summer cycle (C1), which might otherwise be considered as the establishment period, treatment combinations of S1 at T2 and S6 at T1 and T3 produced the greatest yields (Figure 4.12). This data is of particular importance because it identifies the species which are capable of rapid and effective establishment. From a production point of view this is an extremely sought after characteristic as it infers proficiency from the first harvest instead of only from the second or third year of production. It should be noted that S1 seems to have proven to be a well matched substitute for S2 on a biomass basis, as was expected by Jank et al. (2013).

4.5 Calorific values

Calorific value along with annual dry matter yield, are the two most important parameters with respect to bioenergy production. The calorific value indicates the total combustible energy available per unit mass of each respective species.

Due to budget constraints, calorific values were not determined for each harvest interval of each cycle. Instead, a sample of each replication of harvest 3 of cycle 1 was pooled and the calorific value of each species at each water treatment was determined for the pooled sample. Since results were attained from pooled samples, statistical analysis could not be done for this data. However, results will be discussed in a similar fashion to the previous sections. The calorific values, as well as the average calorific value of each species across all three water treatments are presented in Table 4.14.

According to literature there is very little variation between the calorific values of different grass species (Table 2.2). This trial further substantiates these findings since there is a range of merely 1.97 MJ kg^{-1} between the minimum and maximum calorific values across all species (Table 4.14). Furthermore, the calorific value differences within each species only varied between 0.09 - 0.94 MJ kg^{-1} across the three water treatments. The calorific values available in literature for the test species, (Table 2.2), were also quite similar to those attained in the present trial. It is therefore also assumed that calorific value variations will be minimal throughout different growth seasons of the year. Thus, these values were used to determine the total energy production of each cycle, hence, also the total annual energy production.

Guinea grass (S1), S4, S5, S6 and S9 produced their highest calorific value at T1, intermediate at T2 and the lowest value at T3. Napier (S2), S7 and S8 varied with respect to which water treatments produced the greatest and lowest calorific values. It is interesting to note that the majority of species produced their greatest calorific values at the dryland water treatment, and the lowest value at the water treatment with the highest water level, T3. Even though statistical differences could not be determined, these are very positive results with respect to dryland bioenergy production.

Since the calorific value variations within each species were very low, the average value attained for each species was used to compare species against each other. The average calorific value of each species, from highest to lowest, were as follows; S4 (16.75 MJ kg^{-1}), S6 (16.66 MJ kg^{-1}), S5 (16.40 MJ kg^{-1}), S9 (16.25 MJ kg^{-1}), S8 (16.13 MJ kg^{-1}), S1 (15.97 MJ kg^{-1}), S7 (15.74 MJ kg^{-1}) and S2 (15.46 MJ kg^{-1}). Only the top four species; S4, S6, S5 and S9 were able to produce values greater than the overall average across all species, of 16.17 MJ kg^{-1}.

Table 4.14: Calorific values (MJ kg^{-1}) attained from pooled replications of the final harvest of eight *Poaceae* species (S) exposed to three water treatments (T) during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015.

Species	Water treatments			Average
	T1	T2	T3	
S1	16.45	15.95	15.51	15.97
S2	15.61	15.11	15.66	15.46
S4	17.08	16.73	16.45	16.75
S5	16.75	16.25	16.21	16.4
S6	16.94	16.82	16.22	16.66
S7	15.41	16.05	15.77	15.74
S8	16.5	15.84	16.06	16.13
S9	16.29	16.26	16.19	16.25
Average	16.38	16.13	16.01	**16.17**

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.6 Total annual energy yield

Annual energy yield, the single most important parameter with respect to bioenergy production, was determined by multiplying the annual dry matter yield of each treatment combination with its respective calorific value.

4.6.1 Cumulative energy yield of cycles 1, 2 and 3

Statistical analyses could also not be performed on this data since annual energy yield was calculated using the calorific values attained from pooled samples.

It is evident that strong similarities exist between annual energy yield (Figure 4.14) and annual dry matter yield (Figure 4.12). It can thus be expected that since very little variation existed between calorific values, similar statistical differences would exist for annual energy yield as compared to annual dry matter yield. However, such an observation is merely an assumption, and therefore results will be discussed in a similar fashion as previous sections.

Most species produced their greatest energy yields at T3, with decreasing yields at T2 and then further again at T1. Generally, species produced notably greater yields at the two irrigation treatments as compared to the dryland water treatment. The only species that produced a greater total annual energy production at the dryland water treatment (T1) as compared to either of the two irrigation treatments (T2 & T3) was S6. However, as discussed in previous sections, it is strongly suspected that S6 experienced difficulty in the establishment of T2 plots during the first cycle. This species was thus unable to make up the ground lost during cycle 1, and therefore T1 produced a greater overall energy yield as compared to T2. The delay in establishment seems to have been rectified by cycle 2 since this species at T2 produced energy values intermediary to that of T1 and T3 in both cycles 2 and 3. As previously stated, it is expected that during the following year of production S6 would have produced increasing yields from T1 through T3 for each production cycle, as was observed for most other species.

The only other species which did not produce an increasing energy yield from T1 through T3 was S1. The relatively large dry matter yield produced at T2 of cycle 1 also resulted in a production gap which could not be closed by T3, even over both cycles 2 and 3. However, in cycle 3 it seems as if equilibrium had been reached between the different water treatments since the lowest energy yield was produced at T1 and the greatest yield at T3.

The mean annual energy yield across all species for each water treatment was as follows; T1 – 255.41 GJ ha^{-1} yr^{-1}, T2 – 353.89 GJ ha^{-1} yr^{-1} and T3 – 391.26 GJ ha^{-1} yr^{-1}. As already stated, statistical analyses could not be done on this data and therefore it is not possible to state whether or not the differences between the water treatments were statistically significant.

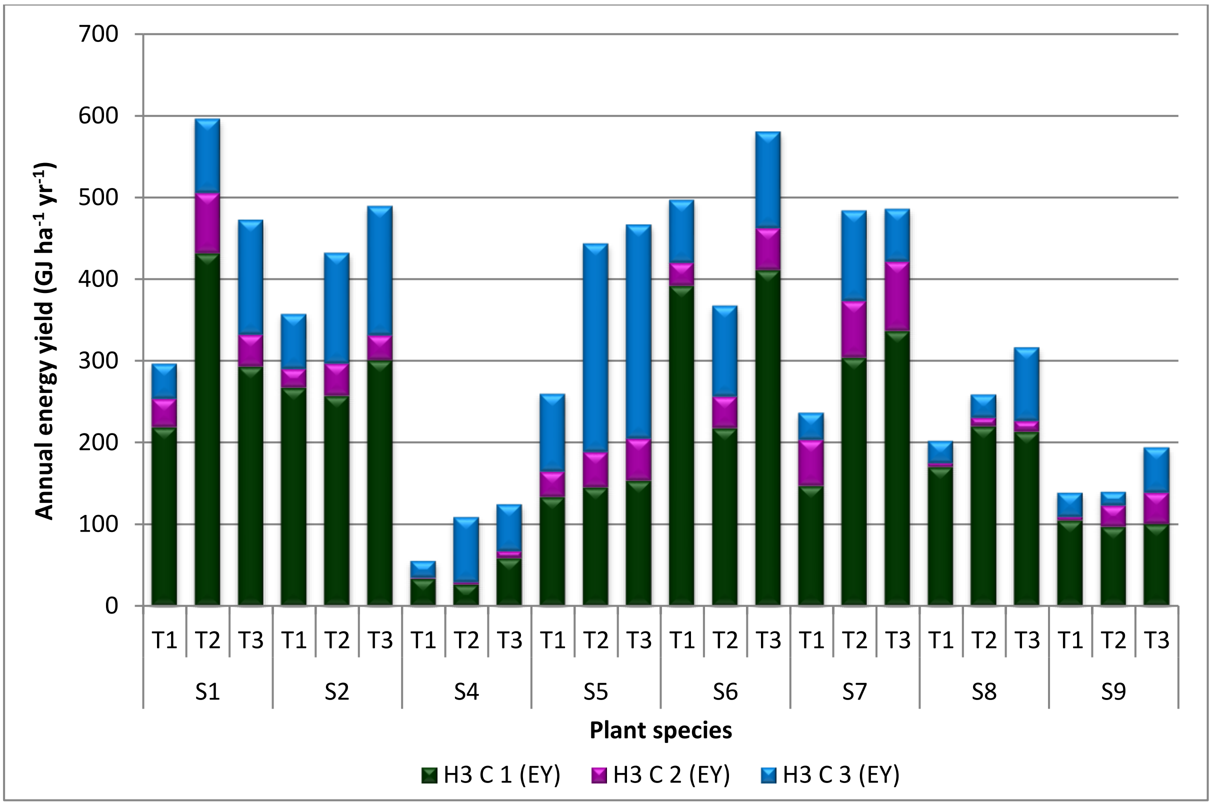


Figure 4.14: Annual energy yield (EY) for the final harvest (H3) of eight *Poaceae* species (S) at each water treatment (T) as stacked energy values for cycle 1 (C1), cycle 2 (C2) and cycle 3 (C3). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

In order to identify which species performed the best on an annual energy yield basis it might make more sense to evaluate the mean production of each species across all three water treatments. In this way species can be analysed across a broader spectrum of water use, thus considering the likelihood of each species to be productive under conditions of varying water supply. Species are listed in ascending order according to mean annual energy yield: S4 (96.23 GJ ha^{-1} yr^{-1}), S9 (157.68 GJ ha^{-1} yr^{-1}), S8 (259.35 GJ ha^{-1} yr^{-1}), S5 (389.88 GJ ha^{-1} yr^{-1}), S7 (402.13 GJ ha^{-1} yr^{-1}), S2 (426.36 GJ ha^{-1} yr^{-1}), S1 (455.03 GJ ha^{-1} yr^{-1}) and S6 (481.49 GJ ha^{-1} yr^{-1}). Only S4 and the two sorghum varieties (S8 & S9) were unable to produce annual energy yields greater than the average across all species, (333.52 GJ ha^{-1} yr^{-1}).

The limited data available in literature regarding the test species suggests that the energy yields attained in the trial were below what was reported for these species. The annual energy yields of only four test species could be obtained from literature, namely for; S2 (548 – 1227 GJ ha^{-1} yr^{-1}) (Khairani et al. 2013, Rengsirikul et al. 2013), S4 (264 – 611 GJ ha^{-1} yr^{-1}) (McLaughlin and Walsh 1998, Ercoli et al. 1999), S5 (1141 – 1956 GJ ha^{-1} yr^{-1}) (Pinners 2014) and S8 (609 GJ ha^{-1} yr^{-1}) (Olivier et al. 2015, Mengistu et al. 2016). The energy yields attained from literature are generally much higher than the energy yields attained for the same species in the present trial.

There may be several reasons for the lower energy yields attained by the test species as compared to the values calculated from literature. For instance, it was not expected that S4 would yield such poor results, especially since this is a species in active use for bioenergy production by countries such as the USA (Erickson et al. 2012). Investigations were thus made into the reason behind this situation and it is suspected that the procedures followed in the process of importing the vegetative material from the USA to South Africa led to severe depletion of carbohydrate reserves within the rhizomes. The vegetative material was reportedly (J van Biljon personal communication 2014) harvested too soon into the growing season, and so, not sufficient time was allowed for the replenishment of reserves metabolized throughout the winter months. An analyses of shoot, root and rhizome reserves that was done on several plant species in the alpine tundra indicated that up to 50% of rhizome reserves can be metabolized within the first week of new season re-growth (Mooney and Billings 1960), which supports the statement above. Thereafter, the rhizomes were imported into South Africa and kept in cold storage for several months until the following season. The propagation material was thus subjected to two successive winter periods, and it is for this reason that this species is suspected to have performed so poorly throughout the trial. It is, therefore, uncertain how accurately the data concerning S4 represents this species on a quantitative as well as a qualitative basis. The most accurate method of attaining reliable data concerning this species would be to re-test it using plant material attained in South Africa, thus avoiding the effects of an additional artificial winter. Much greater energy yields might then be produced from this species.

Vetiver (S5), even at its maximum energy yield attained at T3, produced only a fraction of the energy yield calculated from literature. However, as already addressed in previous sections, it seems that this species not only experienced a delay in establishment, but was also planted at a much lower planting density as what was found to produce optimal dry matter yields. It is, therefore, expected that at higher planting densities and after the second or third season of production, much higher energy yields could be attained.

Sweet sorghum (S8) would have produced a slightly greater dry matter yield had it not been for birds which had eaten the seeds just before the final harvest. Furthermore, the extremely harsh germination and growth conditions of cycle 3 (Figure 4.4) resulted in notably smaller plants as compared to cycle 1, and therefore would also have negatively affected annual energy production.

The maximum annual energy yield produced by S2 was just below the minimum for this species as calculated from literature. It must, however, be kept in mind that all the perennial species tested in the present trial were all analysed in their first year of production. After a second or third year of production it might be expected that stands would have grown to full maturity, and should then again be analysed in order to determine whether energy yields have increased.

4.7 Seasonal and annual water use

In the previous section it was stated that energy yield per annum is arguably the single most important aspect regarding bioenergy crops. However, the water scarcity of a country such as South Africa makes for a compelling reason to determine the water requirements for these crops, and therefore, the seasonal and annual water use of these species are also discussed.

Similar to how fresh matter yield cannot be considered in isolation to determine the bioenergy potential of energy species, so too water use cannot be used in isolation to determine whether or not a species is relatively water demanding. A high water use can be justified if it results in comparatively high energy production, and vice versa. Therefore, a greater deal of focus will be employed in the water use efficiency section.

Water use for each species, either seasonally or annually, is determined by the sum of irrigation water, rainfall and storage, minus drainage and runoff. Storage is the difference between the soil water content at the start of the time interval being measured and the soil water content at the end of that same time interval. Runoff is considered as zero for the present trial since no abnormally large rainfall spells occurred and the terrain was relatively flat. Though it is expected that a certain degree of drainage might have occurred, these values could not be quantified, and thus, for the purpose of the present trial drainage was also considered to be zero. In reality, however, water use values could be expected to be slightly lower than presented in this section.

4.7.1 Production Cycle 1: Dec 2014 – Feb 2015

Only water treatments exhibited significant differences, with the average seasonal water use across all species at T1 (400 mm) and T2 (400 mm) being identical and significantly smaller than at T3 (474 mm) (Table 4.15). It is expected that the similarity between T1 and T2 is due to differences in the success of establishment between different species during the first season of production. The difference in the amount of water applied between T2 and T1 was likely not large enough to make up for factors such as variation in plant size and percentage seed germination across the different species.

Water use values for only four species were found in literature, namely: S2, S4, S8 and S9. However, the “seasonal” periods within which these values were measured for the different species varied between five and 12 months, and thus, were much longer than the 3-month production cycles of the present trial. Therefore, the water use data attained in the present trial will be discussed in greater detail on an annual basis, in section 4.7.4.

The species with the lowest water use values at T1 were S6 (372 mm), S9 (373 mm) and S8 (381 mm). It is not surprising to note that both sorghum varieties ranked in the top three lowest water use values, however, it is interesting to note that S6 had the lowest water use value of all species in the dryland water treatment. Intermediary water use values were exhibited by S1 (389 mm), S5 (390 mm) and S4 (404 mm). The greatest values were recorded for S7 (431 mm) and S2 (454 mm) (Figure 4.15).

The lowest water use values at T2 were recorded for S2 (365 mm), S6 (388 mm) and S4 (394 mm), and it was also only these three species which had water use values lower than the average at T2. Vetiver (405 mm), S8 (407 mm) and S9 (408 mm) were the intermediary species, while S7 (415 mm) and S1 (418 mm) had the highest water use values (Figure 4.15).

Table 4.15: Water use (WU, mm) of eight *Poacea* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (mm)
WU	420	439	420	412	421	441	445	398	425
SEM	17.30	17.30	17.30	17.30	17.30	17.30	17.30	17.30	
LSD	-	-	-	-	-	-	-	-	

Water treatments				
	1	2	3	Average (mm)
WU	400 **a**	400 **a**	474 **b**	425
SEM	12.15	12.15	12.15	
LSD	116.45	116.45	116.45	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM), least significant difference (LSD).

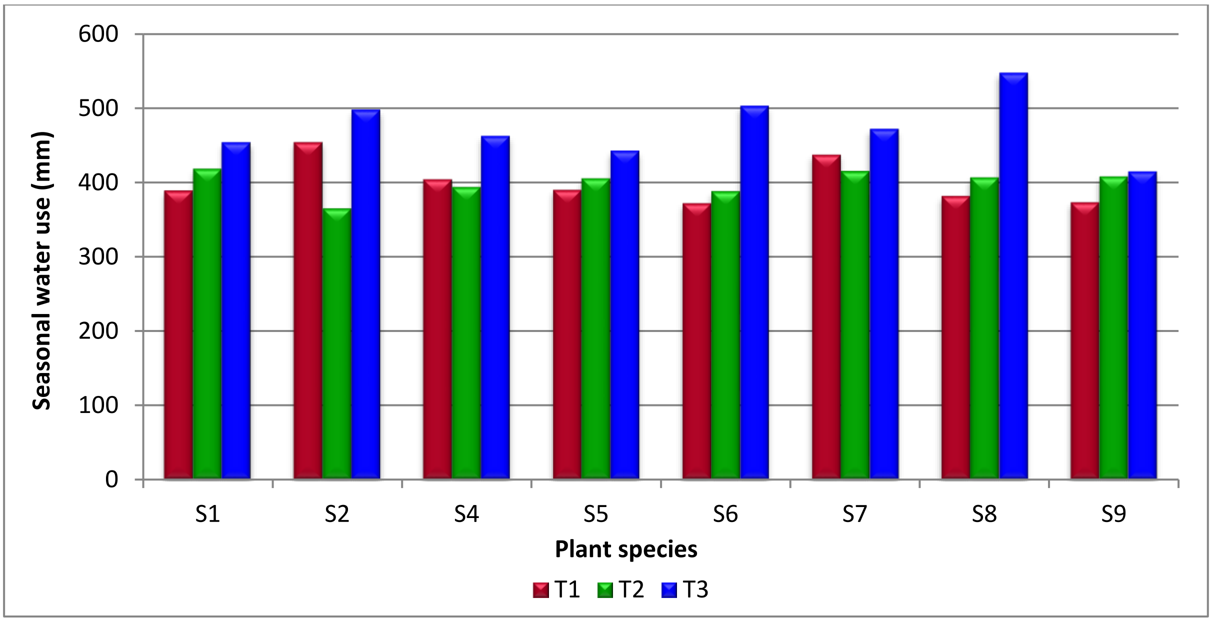


Figure 4.15: Seasonal water use of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Dec 2014 – 28 Feb 2015 (Cycle 1). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Virtually no trend was exhibited for the top three positions across all species and water treatments for the different production cycles, with only S9 regaining the top position at T3. The top three species were; S9 (414 mm), S5 (442 mm) and S1 (454 mm). Intermediate values were recorded for S4 (462 mm), S7 (472 mm) and S2 (498 mm), while the highest water use values were recorded for S6 (503 mm) and S8 (547 mm) (Figure 4.15). At T3 it was again the last three species which had greater water use values than the mean value.

4.7.2 Production Cycle 2: 1 Mar 2015 – 31 May 2015

Significant differences existed between water treatments, species and interactions (Table 4.16).

During production cycle 2 a much clearer effect was observed with respect to variations in water use across the three water treatments, with dryland treatment combinations producing the lowest water use values, followed by those of T2 and T3 (Figure 4.16). The clearer outcome with respect to water use across treatment combinations as compared to that of cycle 1 is likely to be attributed to species being better established in the second cycle. Though all treatment combinations increased in water use with increased water application, only S1 and S7 exhibited a greater increase from T2 to T3, with all other treatment combinations exhibiting a greater increase in water use from T1 to T2 (Figure 4.16).

It might be valuable to note that even though there was a significant interaction, very little variation existed among treatment combinations within each respective water treatment. At T1 and T3 there were no significant differences among treatment combinations, with the exception of S8 at T3, respectively. At T2 only the treatment combinations of S2, S4 and S8 recorded values significantly lower than the other treatment combinations.

Not a single species exhibited treatment combinations which were all below the cycle average (156 mm) at all three water treatments. All dryland treatment combinations and only those of S1, S7 and S8 at T2 exhibited water use values below the cycle average (Table 4.16).

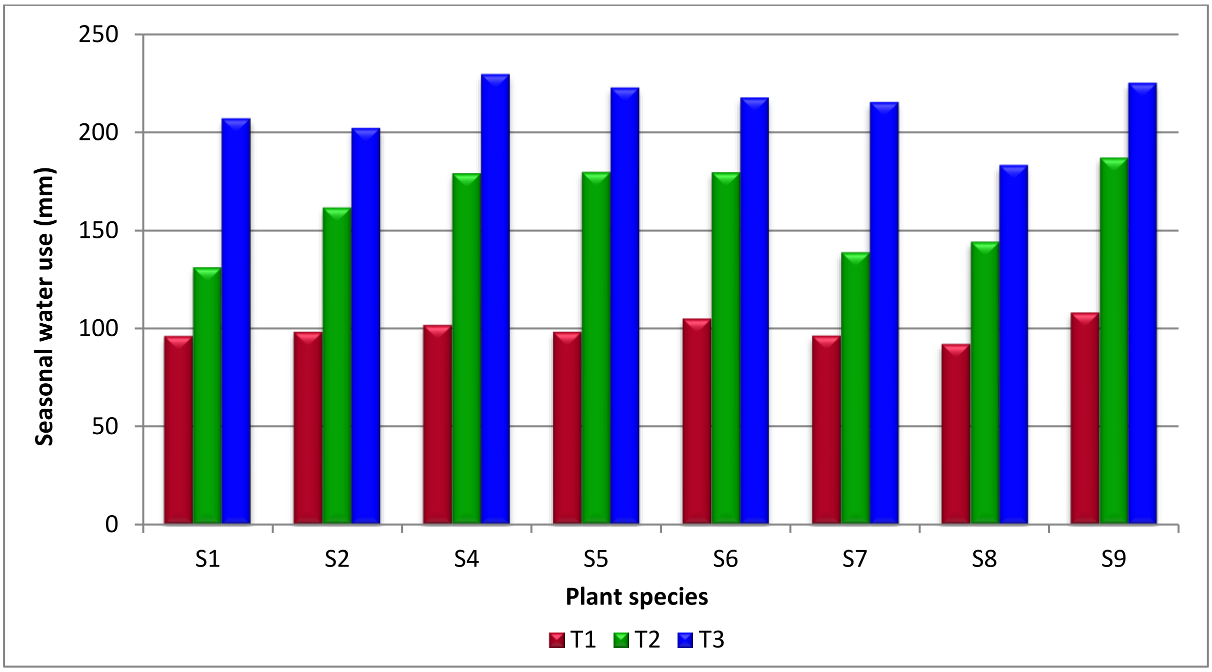


Figure 4.16: Seasonal water use of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Mar 2015 – 31 May 2015 (Cycle 2). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.7.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

Similarly to cycle 1, significant differences existed only for water treatments. The lowest average seasonal water use was recorded at T1 (146 mm), which was significantly similar to T2 (293 mm), but significantly different to T3 (335 mm) (Table 4.17).

At the dryland water treatment S4 (114 mm), S7 (125 mm), S1 (126 mm), S2 (131 mm) and S6 (135 mm) all recorded values lower than the average for this cycle, (146 mm). The remaining species; S8 (154 mm), S5 (176 mm) and S9 (204 mm) all recorded values greater than the average water use at T1 (Figure 4.17).

Table 4.16: Water use (WU, mm) of eight *Poacea* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (mm)	SEM
1	96 **ab**	98 **ab**	102 **ab**	98 **ab**	105 **abc**	96 **ab**	92 **a**	108 **ab**	99	4.22
2	131 **cd**	162 **ef**	179 **fg**	180 **fgh**	179 **fg**	139 **de**	144 **de**	187 **fgh**	163	4.22
3	207 **ghi**	202 **ghi**	229 **i**	223 **i**	217 **i**	215 **i**	182 **fgh**	225 **i**	213	4.22
Average (mm)	145	154	170	167	167	150	139	173	**156**	
SEM	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

Table 4.17: Water use (WU, mm) of eight *Poacea* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (mm)
WU	256	267	240	270	246	236	263	285	258
SEM	16.39	16.39	16.39	16.39	16.39	16.39	16.39	16.39	
LSD	-	-	-	-	-	-	-	-	

Water treatments				
	1	2	3	Average (mm)
WU	146 **a**	293 **a**	335 **b**	258
SEM	16.40	16.40	16.40	
LSD	157.18	157.18	157.18	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM), least significant difference (LSD).

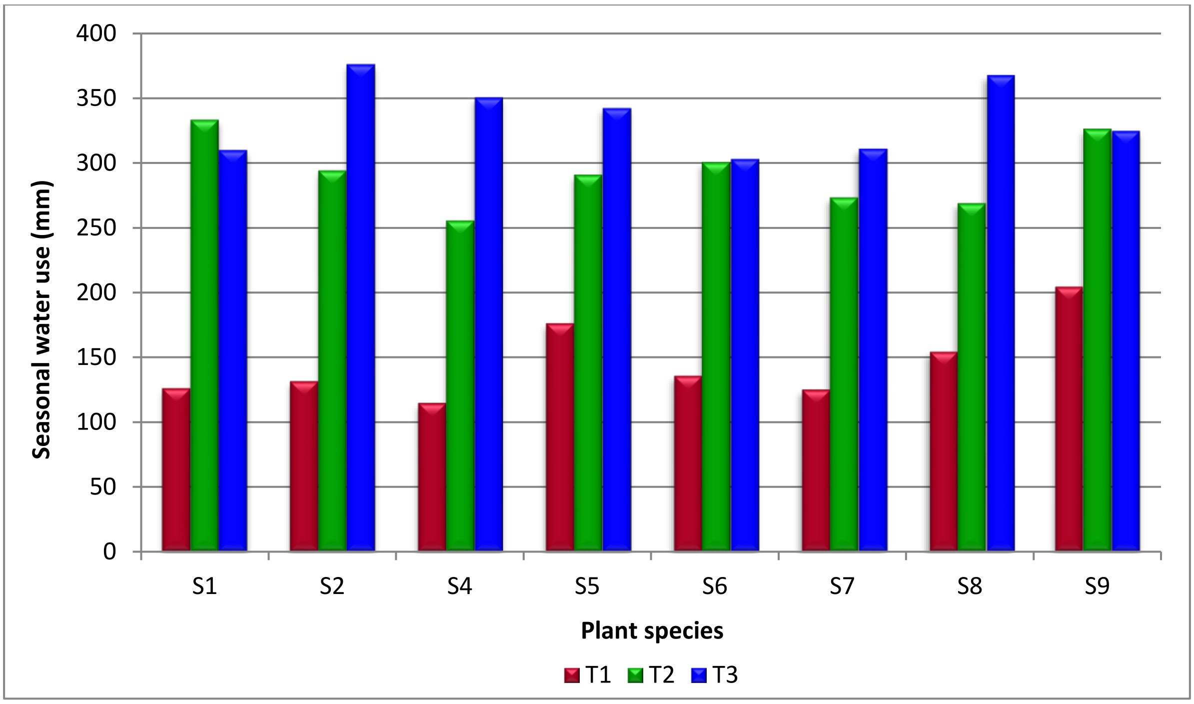


Figure 4.17: Seasonal water use of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Sep 2015 – 30 Nov 2015 (Cycle 3). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Miscanthus (S4 - 225 mm) and S7 (273 mm) were the only species which recorded greater values than the average at T1 as well as T2. In addition to these two species S8 (269 mm) and S5 (291 mm) also recorded values lower than the average at T2, (293 mm). The remaining species were S2 (294 mm), S6 (300 mm), S9 (326 mm) and S1 (333 mm) (Figure 4.17).

Of the four species which recorded water use values lower than the average at T3, (335 mm), namely; S6 (303 mm), S1 (310 mm), S7 (311 mm) and S9 (325 mm), only S7 recorded values lower than the average at all three water treatments. Vetiver (S5 – 342 mm), S4 (350 mm), S8 (367 mm) and S2 (376 mm) recorded values greater than the average at T3.

4.7.4 Annual production: 1 Dec 2014 – 30 Nov 2015

Very little semblance of a trend was established from cycle 1 through cycle 3. Therefore, discussing water use across the different water treatments on an annual basis might make more sense, giving a better summary of the water requirements of each species. Furthermore, the ultimate goal is to determine the potential energy yield and water requirements of each species from an annual point of view.

T1 (634 mm) recorded the lowest average water use across all species, which was also significantly different to T2 (842 mm) and also to T3 (998 mm) (Table 4.18). These results are either on par with or lower than water use values attained in literature for popular bioenergy species such as sugarcane, energy cane and giant reed (Olivier et al. 2015, Triana et al. 2015). It might be valuable to note that interactions were not statistically

Table 4.18: Water use (WU, mm) of eight *Poacea* species exposed to three water treatments from 1 Dec 2014 – 30 Nov 2015 (Annual production).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (mm)
WU	815	826	838	817	832	836	821	814	825
SEM	16.96	16.96	16.96	16.96	16.96	16.96	16.96	16.96	
LSD	-	-	-	-	-	-	-	-	

Water treatments				
	1	2	3	Average (mm)
WU	634 **a**	842 **b**	998 **c**	825
SEM	11.25	11.25	11.25	
LSD	107.82	107.82	107.82	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM), least significant difference (LSD).

significant on an annual basis, indicating that species responded similarly to different water regimes. This might be valuable data when considering the management of these species on a large scale operation. The majority of species recorded lower water use values than the average at T1 (634 mm), namely; S6 (599 mm), S8 (616 mm), S5 (620 mm), S1 (624 mm) and S9 (625 mm). Miscanthus (S4 – 636 mm) was only just above the average, however, S7 (666 mm) and S2 (690 mm) were rather convincingly above the average (Figure 4.18). When observed annually, the two sorghum varieties were still unable to meet the expectations set forward by literature, however, they were at least able to record water use values lower than the average at T1.

Only two species were able to record water use values lower than the average across T2 (842 mm), namely; S2 (779 mm) and S8 (820 mm). Grain sorghum (S9 – 845 mm) and S5 (850 mm) had values fairly close to the average and were followed by S7 (856 mm), S4 (859 mm), S6 (860 mm) and S1 (868 mm) (Figure 4.18). The lowest water use values, and also those below the average at T3 read as follows; S1 (955 mm), S5 (982 mm), S9 (970 mm) and S7 (987 mm). The remaining four species all recorded water use values greater than 1000 mm per annum and read as follows; S2 (1008 mm), S4 (1019 mm), S8 (1028 mm) and S6 (1038 mm) (Figure 4.18).

The only species which was observed in the top four lowest annual water use values across all three water treatments was S5. Thereafter, the only species recurring in the top four within two of the three water treatments were S1, S8 and S9. Though these are positive results in the sense that they indicate relatively low water use values for these species, these values must first be coupled with dry matter production in order to reveal more relevant data – water use efficiency.

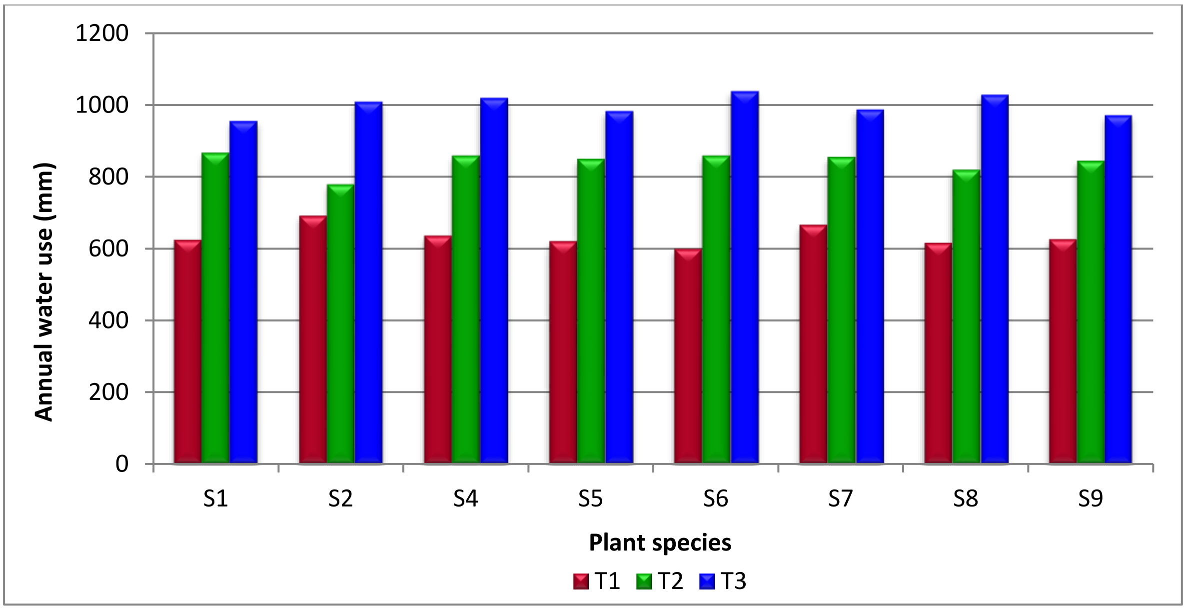


Figure 4.18: Annual water use of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Dec 2014 – 30 Nov 2015. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.8 Seasonal and annual water use efficiency

Water use efficiency (WUE) is calculated by dividing the dry matter yield per hectare of each species by its respective water use. This is extremely relevant data with respect to bioenergy production because it indicates the proficiency with which water is used to produce biomass. The pros and cons of higher water requirements can then be weighed against the production gains delivered by each species under different production conditions.

4.8.1 Production Cycle 1: Dec 2014 – Feb 2015

Only species and interactions produced significant differences. T1 (28.14 kg ha^{-1} mm^{-1}) produced the lowest WUE value, followed by T3 (30.49 kg ha^{-1} mm^{-1}), resulting in T2 (33.08 kg ha^{-1} mm^{-1}) producing the highest average across all water treatments (Table 4.19). The relevance of WUE as a bioenergy parameter is clearly substantiated by these results, indicating that more water does not necessarily lead to improved production.

The massive dry matter yields attained by treatment combination S1 at T2 unsurprisingly resulted in the highest WUE value (64.30 kg ha^{-1} mm^{-1}). All treatment combinations of S6 (T1 – 62.27, T2 – 47.45 & T3 – 50.10 kg ha^{-1} mm^{-1}), and S2 at T2 (46.59 kg ha^{-1} mm^{-1}) were significantly similar to that of S1 at T2. All treatment combinations of S1 (T1 – 34.05, T2 – 64.30 & T3 – 45.12 kg ha^{-1} mm^{-1}), S2 (T1 – 37.54, T2 – 47.67 & T3 – 35.75 kg ha^{-1} mm^{-1}) and S6 (T1 – 62.27, T2 – 47.45 & T3 – 50.10 kg ha^{-1} mm^{-1}) were all above the cycle average. Treatment combinations of S7 at T2 and T3 (T2 – 36.13 & T3 – 39.43 kg ha^{-1} mm^{-1}) and S8 at T2 (34.10 kg ha^{-1} mm^{-1}) were also greater than the cycle average (Table 4.19).

Only the treatment combinations of S5 (T1 – 16.09, T2 – 18.00 & T3 – 21.43 kg ha^{-1} mm^{-1}) and S7 (T1 – 18.90, T2 – 36.13 & T3 – 39.43 kg ha^{-1} mm^{-1}) followed the generally expected trend of increased WUE with increased water application. Of the remaining treatment combinations, S1 (64.30 kg ha^{-1} mm^{-1}), S2 (47.67 kg ha^{-1} mm^{-1}) and S8 (34.10 kg ha^{-1} mm^{-1}) produced their greatest values at T2, S6 (62.27 kg ha^{-1} mm^{-1}) and S9 (17.28 kg ha^{-1} mm^{-1}) at T1 and only S4 (7.92 kg ha^{-1} mm^{-1}) at T3. These results indicate that there is a slight tedancy of treatment combinations to gravitate towards increased water application.

Data attained from WUE values are crucial as it indicates which treatment combinations are most proficient. From this data reliable choices can thus be made regarding species seletion for different regions of South Africa.

The statement made regarding the fact that water use alone is an inconclusive parameter with respect to determining the feasibility of a species as a bioenergy crop, is substantiated in Figure 4.19. From a water use perspective the treatment combinations of both sorghum varieties ranked as intermediary, however, from a WUE perspective the treatment combinations of S9 are consistently ranked among the poorest across all water treatments of this cycle.

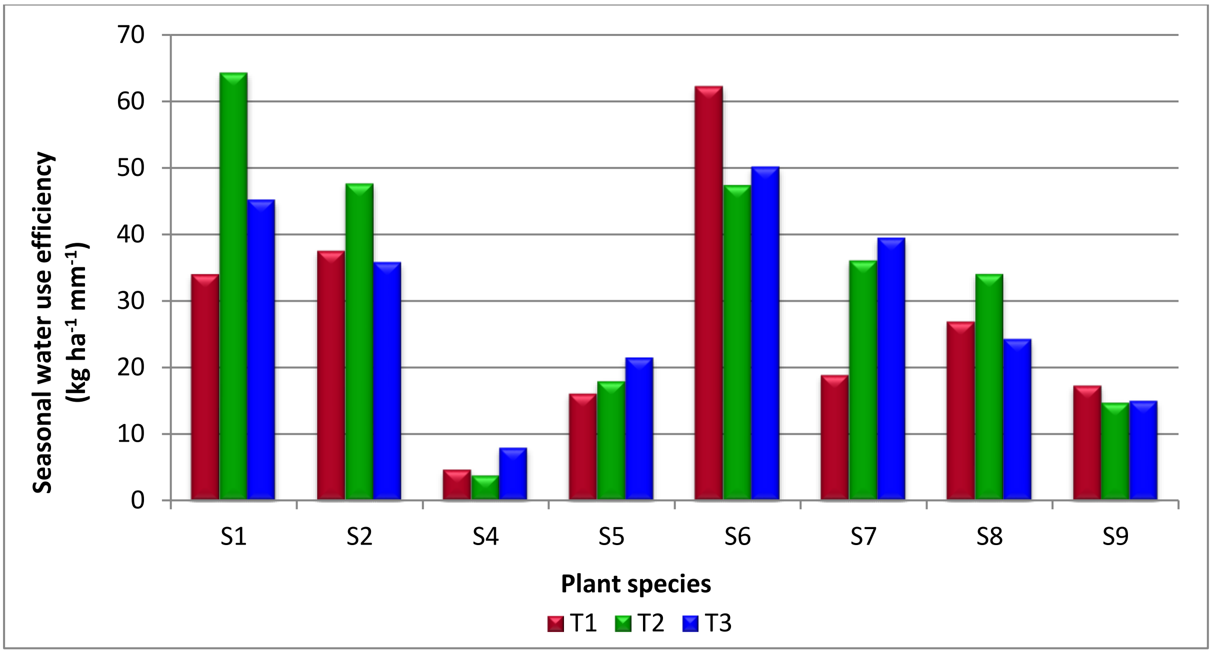


Figure 4.19: Seasonal water use efficiency of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Dec 2014 – 28 Feb 2015 (Cycle 1). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.8.2 Production Cycle 2: 1 Mar 2015 – 31 May 2015

Again an interesting result was observed with T2 (15.33 kg ha^{-1} mm^{-1}) producing the highest average WUE value across all species, followed by T1 (14.65 kg ha^{-1} mm^{-1}) and then by the water treatment which received the most water, T3 (11.01 kg ha^{-1} mm^{-1}). However, only species produced significant differences (Table 4.20).

The highest average WUE values across all water treatments were produced by S7 (T1 – 29.68 kg ha^{-1} mm^{-1}) and S1 (T2 – 23.34 kg ha^{-1} mm^{-1}), both of which were significantly similar to each other. Vetiver (S5 – 16.29 kg ha^{-1} mm^{-1}), S6 (14.45 kg ha^{-1} mm^{-1}) and S2 (13.59 kg ha^{-1} mm^{-1}) produced intermediary values which were all significantly similar to each other as well as to S1. All the above-mentioned species, except for S2, produced values greater than the cycle average, (13.66 kg ha^{-1} mm^{-1}). The remaining species were all significantly similar to each other, namely; S9 (6.57 kg ha^{-1} mm^{-1}), S8 (4.04 kg ha^{-1} mm^{-1}) and S4 (1.36 kg ha^{-1} mm^{-1}) (Table 4.20). It would be valuable to note that the last three species continuously recurred at the lowest positions at all three water treatments in both cycles 1 and 2 (Figures 4.19 and 4.20).

Table 4.19: Water use efficiency (WUE, kg ha^{-1} mm^{-1}) of eight *Poaceae* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (kg ha^{-1} mm^{-1})	SEM
1	34.05 **cdefghijk**	37.54 **cdefgh**	4.62 **m**	16.09 **klm**	62.27 **ab**	18.90 **hijklm**	26.91 **ghijkl**	17.28 **ijklm**	27.21	3.33
2	64.30 **a**	47.67 **abcd**	3.78 **m**	18.00 **ijklm**	47.45 **abcde**	36.13 **cdefghi**	34.10 **cdefghijk**	14.80 **lm**	33.28	3.33
3	45.12 **bcdef**	35.75 **defghij**	7.92 **m**	21.43 **ghijklm**	50.10 **ab**	39.43 **cdefg**	24.23 **ghijkl**	14.94 **lm**	29.87	3.33
Average (kg ha^{-1} mm^{-1})	47.82	40.32	5.44	18.51	53.27	31.49	28.41	15.67	**30.12**	
SEM	3.62	3.62	3.62	3.62	3.62	3.62	3.62	3.62		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

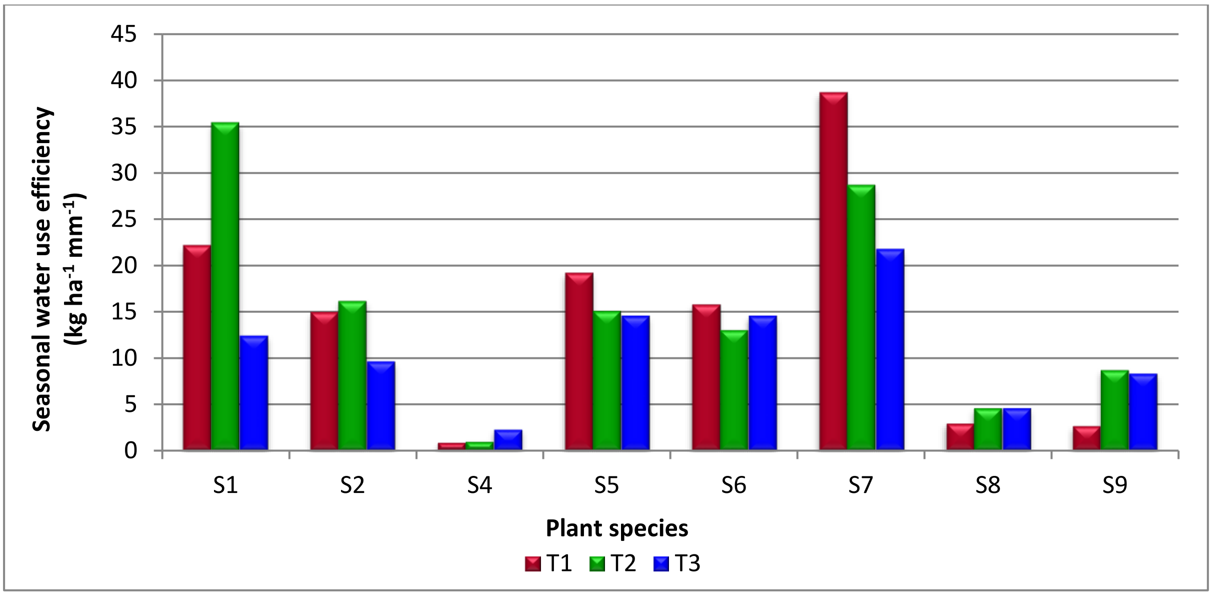


Figure 4.20: Seasonal water use efficiency of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Mar 2015 – 31 May 2015 (Cycle 2). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.8.3 Production Cycle 3: 1 Sep 2015 – 30 Nov 2015

Again only species produced significant differences, however, in this cycle T3 (23.55 kg ha^{-1} mm^{-1}) produce the highest average WUE value across all species, followed by T2 (22.13 kg ha^{-1} mm^{-1}) and T1 (21.27 kg ha^{-1} mm^{-1}) (Table 4.21).

Vetiver (S5 – 45.63 kg ha^{-1} mm^{-1}) produced an average water use efficiency value across all water treatments which was roughly 50% greater than the next best species, and thus was also significantly different to all other species. The next best values were produced by S2 (30.98 kg ha^{-1} mm^{-1}), S6 (29.78 kg ha^{-1} mm^{-1}) and S1 (22.38 kg ha^{-1} mm^{-1}), all of which were significantly similar to each other. The remaining species, namely; S7 (17.76 kg ha^{-1} mm^{-1}), S4 (13.65 kg ha^{-1} mm^{-1}), S8 (11.49 kg ha^{-1} mm^{-1}) and S9 (6.87 kg ha^{-1} mm^{-1}) were all significantly similar to each other and also below the average across all species (22.32 kg ha^{-1} mm^{-1}).

It should be noted that treatment combinations of S5 consistently improved in ranking from cycle 1 to the point where it occupied both first place positions at water treatments T2 and T3 of cycle 3, suggesting that this species might outperform all species in successive future production cycles (Figure 4.24).

Table 4.20: Water use efficiency (WUE, kg ha^{-1} mm^{-1}) of eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (kg ha^{-1} mm^{-1})
WUE	23.34 **ab**	13.59 **bcd**	1.36 **e**	16.29 **bc**	14.45 **bc**	29.68 **a**	4.04 **de**	6.57 **cde**	13.67
SEM	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	
LSD	23.62	23.62	23.62	23.62	23.62	23.62	23.62	23.62	

Water treatments				
	1	2	3	Average (kg ha^{-1} mm^{-1})
WUE	14.65	15.33	11.01	13.66
SEM	1.57	1.57	1.57	
LSD	-	-	-	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM), least significant difference (LSD).

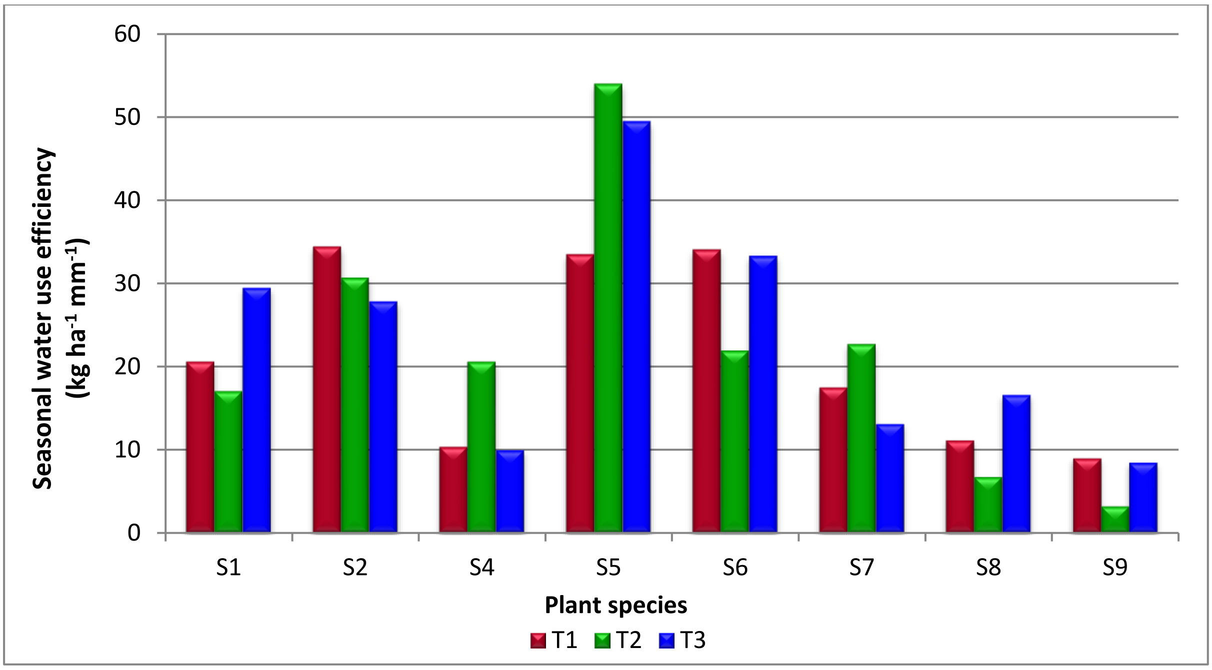


Figure 4.21: Seasonal water use efficiency of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Sep 2015 – 30 Nov 2015 (Cycle 3). Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.8.4 Annual production: 1 Dec 2014 – 30 Nov 2015

Several WUE trends and interesting observations were noted for the three individual production cycles. However, since bioenergy crop production will more often than not be practiced over the full growing season of each year and generally with the intention of perennial production, annual WUE analysis might give more conclusive indications of what can be expected of each species on this front.

Species and interactions produced significantly different values, but once again important results were observed from a water treatment perspective. The lowest average value across all species was produced at T1 (24.02 kg ha^{-1} mm^{-1}), followed by T3 (24.25 kg ha^{-1} mm^{-1}) and T2 (26.11 kg ha^{-1} mm^{-1}) (Table 4.22). The value of these results are once again effectively illustrated by the comparison between T1 and T3, where the application of an additional 370 mm of water, (Figure 4.1), resulted in only a very small difference in water use efficiency of the two water treatments. These results, coupled with the significant differences amongst species emphasize that careful considerations should be made in terms of which species are to be selected for bioenergy production.

Table 4.21: Water use efficiency (WUE, kg ha^{-1} mm^{-1}) of eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3).

Species									
	S1	S2	S4	S5	S6	S7	S8	S9	Average (kg ha^{-1} mm^{-1})
WUE	22.38 **bcd**	30.98 **b**	13.65 **de**	45.63 **a**	29.78 **bc**	17.76 **cde**	11.49 **de**	6.87 **e**	22.32
SEM	3.47	3.47	3.47	3.47	3.47	3.47	3.47	3.47	
LSD	16.06	16.06	16.06	16.06	16.06	16.06	16.06	16.06	

Water treatments				
	1	2	3	Average (kg ha^{-1} mm^{-1})
WUE	21.30	22.10	23.60	22.32
SEM	3.56	3.56	3.56	
LSD	-	-	-	

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM), least significant difference (LSD).

Treatment combinations of S6 at T1 (48.86 kg ha^{-1} mm^{-1}), S1 at T2 (43.11 kg ha^{-1} mm^{-1}) and S2 at T2 (36.86 kg ha^{-1} mm^{-1}) produced the greatest average WUE values, all of which were significantly similar. Concerning S6, these results are invaluable with respect to the literature pool since no data could be found regarding the bioenergy potential of this species. Treatment combinations of the same three species produce the next highest values, all of which were significantly similar to S1 at T2, namely; S6 at T3 and T2 (T3 – 36.11 & T2 – 32.18 kg ha^{-1} mm^{-1}), S1 at T3 (33.33 kg ha^{-1} mm^{-1}) and S2 at T1 (33.27 kg ha^{-1} mm^{-1}) (Figure 4.22). These results clearly identify these three species as strong candidates for bioenergy selection.

Only treatment combinations of S1 (T1 – 28.83, T2 – 43.11 & T3 – 33.33 kg ha^{-1} mm^{-1}), S2 (T1 – 33.27, T2 – 36.86 & T3 – 29.43 kg ha^{-1} mm^{-1}) and S6 (T1 – 48.86, T2 – 32.18 & T3 – 36.11 kg ha^{-1} mm^{-1}) were all above the annual average (24.80 kg ha^{-1} mm^{-1}), and all treatment combinations of S4 (T1 – 4.98, T2 – 7.43 & T3 – 7.53 kg ha^{-1} mm^{-1}), S8 (T1 – 19.87, T2 – 19.96 & T3 – 19.19 kg ha^{-1} mm^{-1}) and 9 (T1 – 13.65, T2 – 10.27 & T3 – 11.20 kg ha^{-1} mm^{-1}) were below the annual average (Figure 4.22).

It is interesting to note that only the treatment combinations of S4 (T1 – 4.98, T2 – 7.43 & T3 – 7.53 kg ha^{-1} mm^{-1}) exhibited increasing WUE values with increasing water application. Treatment combinations of S1 (43.11 kg ha^{-1} mm^{-1}), S2 (36.86 kg ha^{-1} mm^{-1}), S5 (30.00 kg ha^{-1} mm^{-1}), S7 (29.09 kg ha^{-1} mm^{-1}) and S8 (19.96 kg ha^{-1} mm^{-1}) all produced their greatest values at T2, with treatment combinations of S6 (48.86 kg ha^{-1} mm^{-1}) and S9 (13.65 kg ha^{-1} mm^{-1}) producing their greatest values at T1 (Table 4.22).

The data attained regarding WUE values on an annual basis offer very valuable results. It might be concluded that rainfall regions throughout South Africa which most closely resemble that of T2, are the most versatile with respect to bioenergy species selection. These results would, however, be more concrete if the trial was extended to a two or three year period, thus including the longevity of different species into the selection process.

It should be noted that only treatment combinations of S6 produced the greatest WUE value at both T1 and T3, yet at T2 S6 only manage to produce a respective WUE value in third place. It is suspected that under conditions of improved establishment at this water treatment this species would have produced substantially better WUE values at T2 (Figure 4.22).

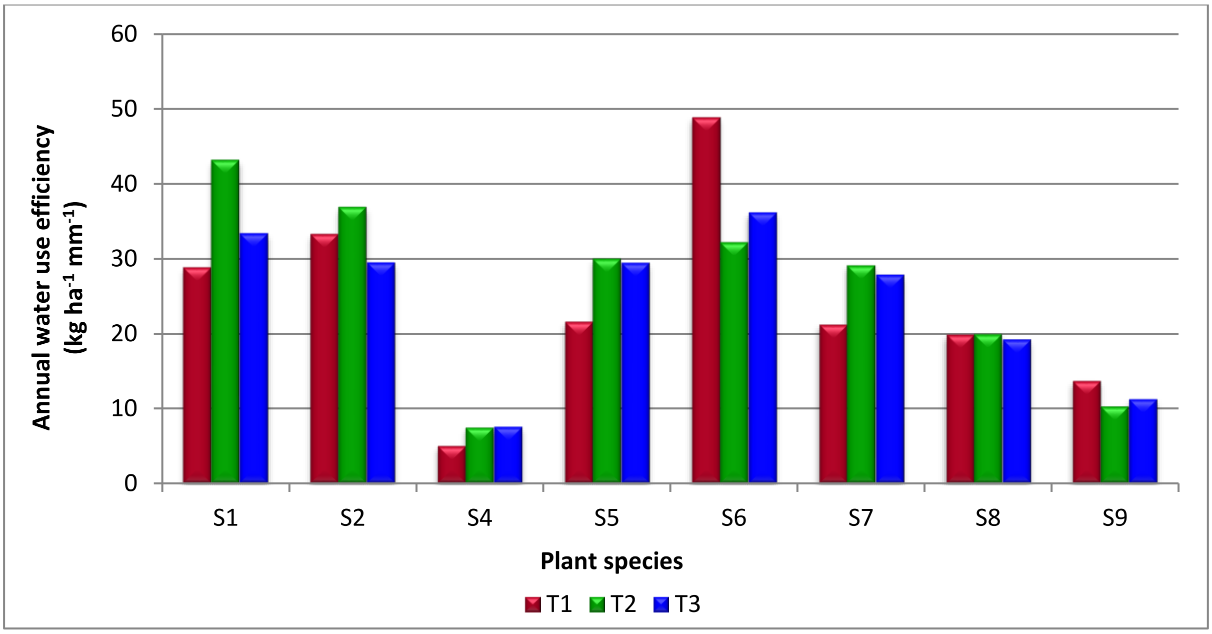


Figure 4.22: Annual water use efficiency of eight potential bioenergy sub-tropical *Poaceae* species (S) at three different water treatments (T) from 1 Dec 2014 – 30 Nov 2015. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

4.9 Annual water to energy production efficiency (WEPE)

Water use efficiency data is extremely relevant with respect to bioenergy production because it indicates the proficiency with which water is used to produce biomass. The pros and cons of higher water requirements can then be weighed against the production gains delivered by each species under different production conditions. However, perhaps the most relevant information with respect to bioenergy production would be a parameter which incorporates energy production instead of dry matter production into the water use efficiency equation. This approach would amalgamate all three of the most important aspects of bioenergy production: dry matter yield, biomass quality (calorific value) and water use efficiency. Such a parameter might already exist, however, nothing bearing any similarity could be found in literature, and therefore, this parameter will be implemented in the present study as water to energy production efficiency (WEPE). For the purpose of this study WEPE was calculated by dividing annual energy yield by the annual water use for each respective treatment combination.

Similar to the trend observed in sections 4.7 and 4.8, the greatest average value across all species was produced at T2 (415 MJ ha^{-1} mm^{-1}), followed by T1 (398 MJ ha^{-1} mm^{-1}) and T3 (384 MJ ha^{-1} mm^{-1}).

Table 4.22: Water use efficiency (WUE, kg ha^{-1} mm^{-1}) of eight *Poaceae* species exposed to three water treatments from 1 Dec 2014 – 30 Nov 2015 (Annual production).

Species										
Water treatments	S1	S2	S4	S5	S6	S7	S8	S9	Average (kg ha^{-1} mm^{-1})	SEM
1	28.83 **cdef**	33.27 **bcd**	4.98 **i**	21.55 **defg**	48.86 **a**	21.18 **defg**	19.87 **efgh**	13.65 **ghi**	24.02	2.46
2	43.11 **ab**	36.86 **abc**	7.43 **hi**	30.00 **cdef**	32.18 **bcde**	29.09 **cdef**	19.96 **efgh**	10.27 **ghi**	26.11	2.46
3	33.33 **bcd**	29.42 **cdef**	7.53 **hi**	29.40 **cdef**	36.11 **bc**	27.81 **cdef**	19.19 **fgh**	11.2 **ghi**	24.25	2.46
Average (kg ha^{-1} mm^{-1})	35.09	33.18	6.65	26.98	39.05	26.03	19.67	11.71	**24.80**	
SEM	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50		

Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum). Standard error of means (SEM).

The greatest value at T1, and also across all water treatments, was again produced by S6 (812 MJ ha^{-1} mm^{-1}), followed by S2 (523 MJ ha^{-1} mm^{-1}) and S1 (485 MJ ha^{-1} mm^{-1}), all of which were greater than the average at T1. Vetiver (S5 – 391 MJ ha^{-1} mm^{-1}) and S7 (360 MJ ha^{-1} mm^{-1}) produced intermediary values, followed by S8 (322 MJ ha^{-1} mm^{-1}), S9 (203 MJ ha^{-1} mm^{-1}) and S4 (89 MJ ha^{-1} mm^{-1}) (Figure 4.23).

At T2 the highest WEPE value was produced by S1 (676 MJ ha^{-1} mm^{-1}), trailed by S7 (585 MJ ha^{-1} mm^{-1}) and S2 (527 MJ ha^{-1} mm^{-1}). Vetiver (S5 – 506 MJ ha^{-1} mm^{-1}) again produced an intermediary value, however, at this water treatment it was joined by S6 (423 MJ ha^{-1} mm^{-1}). The same species as at T1 also occupied the last three positions at T2, and were also the only species below the average at this water treatment, namely; S8 (316 MJ ha^{-1} mm^{-1}), S9 (152 MJ ha^{-1} mm^{-1}) and S4 (131 MJ ha^{-1} mm^{-1}) (Figure 4.23).

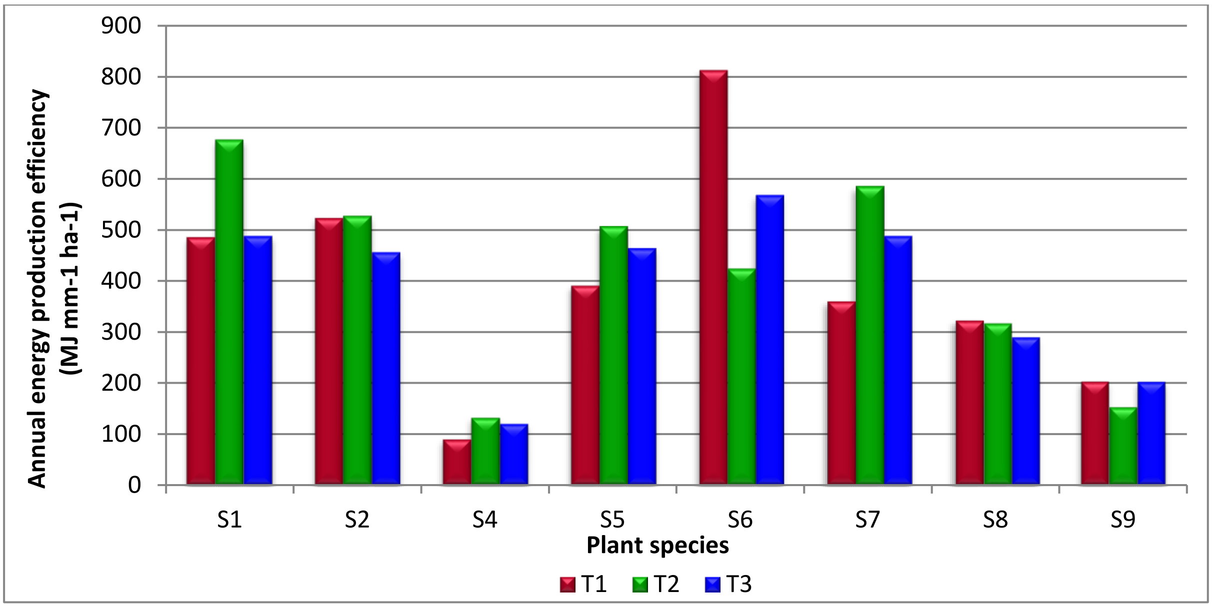


Figure 4.23: Annual water to energy production efficiency (WEPE) for the final harvest (H3) of eight *Poaceae* species (S) at each water treatment (T) from 1 Dec 2014 – 30 Nov 2015. Species: S1 (Guinea grass), S2 (Napier), S4 (Miscanthus), S5 (Vetiver), S6 (Blue thatch), S7 (Brazilian grass), S8 (Sweet sorghum) and S9 (Grain sorghum).

Blue thatch (S6 – 567 MJ ha^{-1} mm^{-1}) once again produced the highest value at T3, followed by four species in close succession to each other, namely; S1 (487 MJ ha^{-1} mm^{-1}), S7 (487 MJ ha^{-1} mm^{-1}), S5 (464 MJ ha^{-1} mm^{-1}) and S2 (455 MJ ha^{-1} mm^{-1}). The same trend exhibited at T1 and T2 was observed at T3, with S8 (289 MJ ha^{-1} mm^{-1}), S9 (202 MJ ha^{-1} mm^{-1}) and S4 (119 MJ ha^{-1} mm^{-1}) being the only species to produce values below the average at this water treatment (Figure 4.23).

The average WEPE value of each species across all water treatments might be the single most valuable value used to indicate the versatility and feasibility of each species across a wide range of different water conditions. In effect these values will determine the best and worst species of those tested, for bioenergy production in South African, specifically under subtropical conditions. Species are ranked in descending order as follows; S6 (601 MJ ha^{-1} mm^{-1}), S1 (549 MJ ha^{-1} mm^{-1}), S2 (502 MJ ha^{-1} mm^{-1}), S7 (477 MJ ha^{-1} mm^{-1}), S5 (454 MJ ha^{-1} mm^{-1}), S8 (309 MJ ha^{-1} mm^{-1}), S9 (185 MJ ha^{-1} mm^{-1}) and S4 (113 MJ ha^{-1} mm^{-1}). This data, and further testing thereof, is crucial since three of the top four species are either lacking or completely void of bioenergy data within the literature pool.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Many factors affect the feasibility of a crop as a potential candidate for proficient, sustainable and renewable bioenergy production. Some of these factors offer direct insight into the expected energy yield of each species, while others might draw attention to areas which might be improved upon by processes such as selective breeding to create varieties best suited for energy production. Throughout the present study compelling evidence was put forward, suggesting that fresh matter yield (FMY), leaf area index (LAI) and dry matter percentage (DM %) cannot be used in isolation to determine the production potential of a bioenergy species. However, valuable data was still attained through the analysis of these parameters.

Generally, the LAI values attained throughout the trial were lower than those available in literature for respective species. The most probable reason for this outcome was that species were analysed in the first year of production. This reasoning only stands with respect to perennial species, however, since 75% of the species tested were perennials, a logical approach would be to reanalyse the LAI values of these species in subsequent years of production. Several subsequent years of analyses would likely also confirm or reject the second theory; that species were subject to above average harsh climatic conditions in the final production cycle. Data was also attained which tends to suggest that alterations in growth cycle length might be beneficial on an annual energy yield basis. In cycle 1 Vetiver (S5), Blue thatch (S6) and Brazilian grass (S7) produced LAI values which seemed to still be increasing at the final harvest of each water treatment. This strongly suggests that these species had not reached maximum canopy size (maturity) by the end of this cycle. Additionally, the generally greater final dry matter yields as compared to the theoretical yields suggest that yields attained at increased maturity are greater than those attained when harvested more often before maturity. Therefore, since a general trend of increasing dry matter yield with increasing maturity exists for these species, it might be worthy to note the effects on dry matter yield if these three species were grown under conditions of increased cycle lengths.

The generally much lower dry matter yields of cycle 3 as compared to cycle 1 are suspected to be attributed to the harsh climatic conditions experienced during cycle 3. If the assumption is made that under “normal” climatic conditions the dry matter yields attained in cycle 3 would be greater than those produced in the present trial, then it might be advisable to have two longer growth cycles instead of three three-month growth cycles. Cycle 1 would then start at the beginning of September and last until the end of December or January, depending on the level of maturity attained by each respective species. Cycle 2 would then start at the beginning of January or February and last until the end of April or May. Any re-growth produced before dormancy would be harvested at the beginning of September, also stimulating the growth of the next cycle. It would, however, be worthwhile to test all perennial species under conditions of two longer growth cycles, not only S5, S6 and S7.

The unanimous dry matter percentage increase from harvest 1 to harvest 3 in cycle 1, and the unanimous decrease from harvest 2 to harvest 3 in cycle 2 strongly support the recommendations of two longer growth cycles, instead of three shorter cycles. When considering all three growth cycles, the majority of highest dry matter percentage values, and also calorific values, were produced at the dryland water treatment. These outcomes could potentially also be exploited by increasing growth cycle lengths, resulting in maximum production efficiency, specifically under dryland conditions.

On a water use basis virtually no trend was established between the different growth cycles, and therefore water use was more stringently focused on from an annual perspective. As was expected water treatments of differing water application were all significantly different to each other, however, a rather unexpected result was that there were no significant differences among species across different water regimes.

From a water use efficiency (WUE) perspective very little difference was observed between water treatments on an annual basis. These results in conjunction with the significant differences observed among species placed emphasis on the fact that careful selection of species needs to be made concerning bioenergy production. The same three species, namely; S6, S1 and S2 occupied the majority of the top three positions across all three water treatments. The influence of WUE on the bioenergy potential of a species is clearly fundamental since these are also the three species rated as the best bioenergy species according to the parameters tested in the present trial.

Water use, dry matter yield and calorific value are all parameters of utmost importance regarding bioenergy production, yet, even these data can give misleading perceptions about species if considered in isolation. It is therefore recommended that these three parameters are combined into a single parameter, water to energy production efficiency (WEPE), in order to provide a full and objective spectrum of each species as potential bioenergy crops. The average WEPE value of each species across all water treatments enabled the identification of the most versatile and feasible bioenergy species under subtropical conditions in South Africa. Blue thatch (S6) is considered to be the best bioenergy species under the present trial conditions, followed by S1 and S2. It is interesting that no literature could be found on S6, and yet, it proved to be the most proficient species among all those tested. Guinea grass (S1) also proved to be a more than competent seeded alternative to S2, comfortably outperforming this species. Brazilian grass (S7) and S5 followed fairly closely behind S2, occupying the intermediary positions.

Perennial bioenergy species should remain productive after many years of production, and therefore, these results cannot be finally concluded until several years of successive tests have been completed. With this in mind, it is strongly suspected that S5 might become the most proficient bioenergy species of those tested, perhaps as early as in the second year of production. The very large dry matter yields and longevity reported in literature, in addition to the continual improvement in ranking from cycle 1 to cycle 3 lay claim to this theory. Sweet sorghum (S8), S9 and S4 however, produced values far lower than all other species, though for S4 this is understandable according to the reasons given in Section 3. It might be said that better results were expected from the sorghum varieties, especially considering the desirable characteristic of comparatively very low water use values reported in literature, however, it must be kept in mind that this species is primarily not grown for biomass production, but rather for grain or feed production. This study further revealed that both S1 and S7 are relatively poor perennials, and thus, these species should perhaps be considered as annual crop rotation species, instead of the rather poor performing sorghum varieties.

Not having specifically measured the stem to leaf ratio of the test species resulted in a rather inconclusive recommendation regarding the desirability of either direction of this ratio. There is also very little semblance of a trend when comparing total average LAI values of each species with respective average annual dry matter yields. Specific studies regarding this characteristic in bioenergy species will have to be undertaken in order to definitively conclude which result is generally the most desirable.

The native advantage and robustness of indigenous species is clearly evident with four out of the five best performing species on a WEPE basis being indigenous species. Besides for S5 in fifth place, the other exotic species, S4, produced the lowest WEPE value of all species tested. The origin or geographical distribution of a species is thus most definitely a factor which should be considered when selecting for bioenergy species.

Three final recommendations have been made concerning the present trial: Firstly, all species should be analysed under conditions of increased cycle length in order to establish whether increased WEPE values could be attained. Secondly, perennials should be analysed for several successive growth seasons in order to determine the production repeatability and thus feasibility of these species as perennial bioenergy crops. Lastly, the most promising species should undergo selective breeding to improve traits such as dry matter production and water use, where after the improved varieties should be analysed under the same parameters as in the present trial.

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APPENDIX

A1 Statistical analysis of eight *Poaceae* species exposed to three water treatments during the summer growth cycle from 1 Dec 2014 – 28 Feb 2015 (Cycle 1)

```
Summer Harvest 3 all Nov2016.rtf

WUE of large biomass grasses: SUMMER HARVEST 3

     Identifier  Minimum     Mean  Maximum   Values  Missing

             FW     1208    42035   113700       72        1

             DW    468.4    12955    33626       72        1

             DM    18.12    32.55    48.24       72        1

            LAI  0.04163    2.241    6.702       72        1

========== REML split-plot factorial analysis on fresh weight with all species ==========

REML variance components analysis

Response variate:  FW

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep + Rep.wplot + Rep.wplot.splot

Number of units:   71 (1 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                 Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                           13.24        2          6.62      4.0   0.054

Species                            633.61        7         90.52     41.0  <0.001

Treatment.Species                  132.71       14          9.48     41.0  <0.001
```

```
Table of predicted means for Treatment

Treatment       1       2       3

            34399   44776   47474

Standard errors

Treatment       1       2       3

             3235    3256    3235

Standard errors

Average:               3242.

Maximum:               3256.

Minimum:               3235.

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

          64681   64252    5866   26492   46350   53248   56810   20029

Standard errors

Species       1       2       4       5       6       7       8       9

           3240    3240    3240    3240    3240    3388    3240    3240

Standard errors

Average:               3259.

Maximum:               3388.

Minimum:               3240.

Table of predicted means for Treatment.Species

    Species       1       2       4       5       6       7       8       9

  Treatment

          1   38008   60867    4301   21546   51257   30530   48567   20115

          2   92133   63150    3973   29568   30072   60485   58696   20128

          3   63901   68738    9325   28362   57722   68730   63167   19844

Standard errors

       Species       1       2       4       5       6       7       8       9
```

```
Treatment
           1   4944   4944   4944   4944   4944   4944   4944   4944
           2   4944   4944   4944   4944   4944   5770   4944   4944
           3   4944   4944   4944   4944   4944   4944   4944   4944
Standard errors
 Average: 4979.
Maximum:              5770.
Minimum:              4944.
  ========== Comparing means at the 5% level =========
Comparisons between Species means
        Mean
1     64681   a
2     64252   a
8     56810   ab
7     53248   bc
6     46350   c
5     26492   d
9     20029   d
4      5866   e
Comparisons between Treatment.Species means
Variances vary and decisions regarding group membership are inconsistent, so there may
be gaps in the lines or letters linking means in identical groups.
          Mean
2 1     92133   a
3 2     68738   ab
3 7     68730   ab
3 1     63901   abc
3 8     63167   abcd
2 2     63150   abcde
```

```
1 2     60867  abcdef
2 7     60485  abcdefg
2 8     58696  abcdefgh
3 6     57722  abcdefghi
1 6     51258  abcdefghij
1 8     48567  bcdefghij
1 1     38008  bcdefghijk
1 7     30530  bcdefghijk
2 6     30072  bcdefghijk
2 5     29568  bcdefghijk
3 5     28362  cdefghijk
1 5     21546  cdeghijk
2 9     20128  dfgijk
1 9     20115  deghijk
3 9     19844  efghijk
3 4      9325  jk
1 4      4301  k
2 4      3973  k
   ============ Summary of data for SDs ===========
      Species         1                      2
             Nobservd      Mean      s.d. Nobservd      Mean      s.d.
    Treatment
            1         3     38008      7561        3     60867      9741
            2         3     92133     21233        3     63150     14856
            3         3     63901      8056        3     68738      3528
       Margin         9     64681     26322        9     64252      9712
      Species         4                      5
             Nobservd      Mean      s.d. Nobservd      Mean      s.d.
```

Treatment						
1	3	4301	3620	3	21546	1482
2	3	3973	4668	3	29568	7215
3	3	9325	7156	3	28362	8496
Margin	9	5866	5317	9	26492	6756

Species	6			7		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	51258	10942	3	30530	2601
2	3	30072	2470	2	63200	14177
3	3	57722	10277	3	68730	7436
Margin	9	46350	14654	8	53022	19965

Species	8			9		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	48567	8300	3	20115	4054
2	3	58696	9699	3	20128	2362
3	3	63167	3991	3	19844	1783
Margin	9	56810	9311	9	20029	2513

Species	Margin		
	Nobservd	Mean	s.d.
Treatment			
1	24	34399	18910
2	23	44329	29434
3	24	47474	23855

```
    Margin         71      42035      24670

========== REML split-plot factorial analysis on dry weight with all species ==========

REML variance components analysis

Response variate:  DW

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep + Rep.wplot + Rep.wplot.splot

Number of units:   71 (1 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model


Fixed term                    Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                               7.17        2          3.58      4.0   0.128

Species                               450.34        7         64.33     41.0  <0.001

Treatment.Species                     107.63       14          7.69     41.0  <0.001

Table of predicted means for Treatment

Treatment       1       2       3

            11201   13165   14644

Standard errors

Treatment       1       2       3

             1076    1084    1076

Standard errors

Average:               1079.

Maximum:               1084.

Minimum:Table of predicted means for Species


Species       1       2       4       5       6       7       8       9
```

```
          19717  17755   2350   8776  20446  16313  12483   6186

 Standard errors

Species       1      2      4      5      6      7      8      9

           1099   1099   1099   1099   1099   1155   1099   1099

Standard errors

Average:              1106.

Maximum:              1155.

Minimum:              1099.

Table of predicted means for Treatment.Species

    Species        1      2      4      5      6      7      8      9

  Treatment

              1  13287  17086   1937   7950  23092   9532  10308   6418

              2  27008  17001   1559   8929  12919  18094  13863   5946

              3  18858  19179   3555   9448  25328  21312  13278   6195

Standard errors

  Species        1      2      4      5      6      7      8      9

 Treatment

            1   1721   1721   1721   1721   1721   1721   1721   1721

            2   1721   1721   1721   1721   1721   2024   1721   1721

            3   1721   1721   1721   1721   1721   1721   1721   1721

Standard errors

Average:              1734.

Maximum:              2024.

Minimum:              1721.


   ========== Comparing means at the 5% level =========

Comparisons between Species means

         Mean
```

```
6      20446   a

1      19717   ab

2      17755   ab

7      16313   b

8      12483   c

5       8776   d

9       6186   d

4       2350   e

Comparisons between Treatment.Species means

Variances vary and decisions regarding group membership are inconsistent, so there may
be gaps in the lines or letters linking means in identical groups.

              Mean

2 1      27008   a

3 6      25328   ab

1 6      23092   abc

3 7      21312   abcd

3 2      19179   abcde

3 1      18858   abcde

2 7      18094   abcdef

1 2      17086   abcdefg

2 2      17001   abcdefgh

2 8      13863   abcdefghi

1 1      13287   abcdefghi

3 8      13278   abcdefghi

2 6      12919   bcdefghi

1 8      10308   bcdefghi

1 7       9532   cdefghi

3 5       9448   cdefghi

2 5       8929   cdefghi
```

```
1 5         7950   defghi

1 9         6418   defghi

3 9         6195   efghi

2 9         5946   defghi

3 4         3555   fghi

1 4         1937   fhi

2 4         1559   i

   ============ Summary of data for SDs ===========

     Species         1                              2

              Nobservd       Mean        s.d.  Nobservd       Mean        s.d.

   Treatment

           1         3      13287        1959         3      17086        3198

           2         3      27008        6498         3      17001        3520

           3         3      18858        2627         3      19179        1495

      Margin         9      19717        6997         9      17755        2712

     Species         4                              5

              Nobservd       Mean        s.d.  Nobservd       Mean        s.d.

   Treatment

           1         3       1937        1711         3       7950         725

           2         3       1559        1820         3       8929        1975

           3         3       3555        2803         3       9448        3520

      Margin         9       2350        2090         9       8776        2154


     Species         6                              7

              Nobservd       Mean        s.d.  Nobservd       Mean        s.d.

   Treatment

           1         3      23092        6049         3       9532         735

           2         3      12919        2078         2      18926        5375
```

```
          3         3     25328      4530          3     21312      1846

     Margin         9     20446      6940          8     16298      6134


    Species         8                              9

             Nobservd      Mean      s.d.   Nobservd      Mean      s.d.

  Treatment

          1         3     10308      1706          3      6418      1551

          2         3     13863      2922          3      5946       633

          3         3     13278      2180          3      6195       434

     Margin         9     12483      2603          9      6186       889


    Species    Margin

             Nobservd      Mean      s.d.

  Treatment

          1        24     11201      6691

          2        23     13023      8202

          3        24     14644      7738

     Margin        71     12955      7587

========== REML split-plot factorial analysis on dry matter % with all species ==========

REML variance components analysis

Response variate:  DM

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep + Rep.wplot + Rep.wplot.splot

Number of units:   71 (1 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model
```

```
Fixed term                    Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                              27.14        2         13.57      4.0   0.016

Species                               709.05        7        101.29     41.2  <0.001

Treatment.Species                      30.91       14          2.21     41.2   0.025

Table of predicted means for Treatment

Treatment        1        2        3

             34.16    31.49    31.90

Standard errors

Treatment        1        2        3

              0.36     0.37     0.36

Standard errors

Average:                0.3647

Maximum:                0.3742

Minimum:                0.3599

Table of predicted means for Species

Species        1        2        4        5        6        7        8        9

           31.33    27.64    40.42    33.36    43.84    30.74    21.90    30.88

Standard errors

Species        1        2        4        5        6        7        8        9

            0.67     0.67     0.67     0.67     0.67     0.72     0.67     0.67

Standard errors

Average:                0.6741

Maximum:                0.7212

Minimum:                0.6674

Table of predicted means for Treatment.Species

   Species        1        2        4        5        6        7        8        9

  Treatment

            1 35.24    27.99    44.08    36.92    44.76    31.24    21.25    31.80
```

```
          2  29.27  27.04  39.50  30.35  42.79  29.88  23.50  29.57

          3  29.49  27.87  37.67  32.82  43.97  31.10  20.96  31.28

Standard errors

  Species      1      2      4      5      6      7      8      9

 Treatment

        1   1.17   1.17   1.17   1.17   1.17   1.17   1.17   1.17

        2   1.17   1.17   1.17   1.17   1.17   1.43   1.17   1.17

        3   1.17   1.17   1.17   1.17   1.17   1.17   1.17   1.17

Standard errors

Average:             1.181

Maximum:             1.429

Minimum:             1.170

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

      Mean

1     34.16  a

3     31.90  b

2     31.49  b

Comparisons between Species means

      Mean

6     43.84  a

4     40.42  b

5     33.36  c

1     31.33  c

9     30.88  c

7     30.74  cd

2     27.64  d

8     21.90  e
```

Comparisons between Treatment.Species means

Note: this happens with the Tukey test!

Fisher's protected LSD - comparisons not calculated as variance ratio for Treatment.Species is not significant.

```
   ============= Summary of data for SDs ============

    Species         1                         2

             Nobservd      Mean      s.d. Nobservd      Mean      s.d.

  Treatment

          1         3     35.24     3.292        3     27.99     0.849

          2         3     29.27     0.305        3     27.04     0.799

          3         3     29.49     0.997        3     27.87     0.769

     Margin         9     31.33     3.402        9     27.64     0.830

    Species         4                         5

             Nobservd      Mean      s.d. Nobservd      Mean      s.d.

  Treatment

          1         3     44.08     2.093        3     36.92     2.794

          2         3     39.50     0.989        3     30.35     2.012

          3         3     37.67     1.670        3     32.82     2.961

     Margin         9     40.42     3.197        9     33.36     3.661


    Species         6                         7

             Nobservd      Mean      s.d. Nobservd      Mean      s.d.

  Treatment

          1         3     44.76     2.322        3     31.24     0.405

          2         3     42.79     3.383        2     29.74     1.833

          3         3     43.97     3.888        3     31.10     2.029

     Margin         9     43.84     2.954        8     30.81     1.465


    Species         8                         9
```

```
              Nobservd       Mean        s.d. Nobservd       Mean        s.d.
   Treatment
           1         3      21.25       0.711        3      31.80       2.113
           2         3      23.50       1.154        3      29.57       0.462
           3         3      20.96       2.473        3      31.28       1.813
      Margin         9      21.90       1.854        9      30.88       1.736

     Species    Margin
              Nobservd       Mean        s.d.
   Treatment
           1        24      34.16       7.756
           2        23      31.54       6.406
           3        24      31.90       6.761
      Margin        71      32.55       7.004
========== REML split-plot factorial analysis on LAI with all species ==========
REML variance components analysis
Response variate:  LAI
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   71 (1 units excluded due to zero weights or missing values)
Rep.wplot.splot used as residual term
 Sparse algorithm with AI optimisation


 Tests for fixed effects
 Sequentially adding terms to fixed model
Fixed term                 Wald statistic   n.d.f.    F statistic    d.d.f.     F pr
Treatment                           24.57        2          12.28       4.0    0.020
Species                            521.45        7          74.49      41.1   <0.001
```

```
Treatment.Species                111.79      14          7.98     41.1 <0.001

 Table of predicted means for Treatment

 Treatment                                   1        2             3

                1.943   2.560   2.339

 Standard errors

 Treatment      1        2        3

                0.104   0.106   0.104

 Standard errors

Average:        0.1047

Maximum:                  0.1064

Minimum:                  0.1038

 Table of predicted means for Species

 Species                         1 2        4        5        6        7        8        9

             4.373   2.841   0.258   1.140   1.885   3.726   2.514   1.508

 Standard errors

 Species      1        2        4        5        6        7        8        9

             0.167   0.167   0.167   0.167   0.167   0.178   0.167   0.167

 Standard errors

 Average: 0.1681

Maximum:                  0.1779

Minimum:                  0.1667

 Table of predicted means for Treatment.Species



Species     1        2        4        5        6        7        8        9

   Treatment

                1   2.790   3.222   0.182   0.854   1.899   2.924   2.187   1.488

                2   6.061   2.761   0.070   1.292   1.147   5.102   2.526   1.524
```

```
             3   4.267   2.539   0.524   1.274   2.610   3.152   2.829   1.513

Standard errors

   Species         1         2         4         5         6         7         8         9

 Treatment

           1   0.270   0.270   0.270   0.270   0.270   0.270   0.270   0.270

           2   0.270   0.270   0.270   0.270   0.270   0.329   0.270   0.270

           3   0.270   0.270   0.270   0.270   0.270   0.270   0.270   0.270

Standard errors

Average:                0.2729

Maximum:                0.3286

Minimum:                0.2705

     ========== Comparing means at the 5% level =========

Comparisons between Treatment means

          Mean

2         2.560   a

3         2.339   a

1         1.943   b

 Comparisons between Species means

          Mean

1         4.373   a

7         3.726   a

2         2.841   b

8         2.514   bc

6         1.885   cd

9         1.508   de

5         1.140   e

4         0.258   f

Comparisons between Treatment.Species means
```

```
              Mean
2 1          6.061   a
2 7          5.102   ab
3 1          4.267   abc
1 2          3.222   bcd
3 7          3.152   bcd
1 7          2.924   bcde
3 8          2.829   bcde
1 1          2.790   bcde
2 2          2.761   bcde
3 6          2.610   bcdef
3 2          2.539   bcdefg
2 8          2.526   bcdefg
1 8          2.187   cdefg
1 6          1.899   cdefg
2 9          1.524   defg
3 9          1.513   defg
1 9          1.488   defg
2 5          1.292   defg
3 5          1.274   defg
2 6          1.147   defg
1 5          0.854   defg
3 4          0.524   efg
1 4          0.182   fg
2 4          0.070   g
    ============ Summary of data for SDs ===========
      Species            1                                 2
                 Nobservd      Mean          s.d.  Nobservd      Mean          s.d.
```

```
 Treatment
         1         3     2.790    0.3834         3     3.222    0.5488
         2         3     6.061    0.9927         3     2.761    0.7229
         3         3     4.267    0.4232         3     2.539    0.5294
    Margin         9     4.373    1.5299         9     2.841    0.6058

   Species         4                             5
            Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
 Treatment
         1         3     0.182    0.1229         3     0.854    0.2167
         2         3     0.070    0.0365         3     1.292    0.4553
         3         3     0.524    0.5305         3     1.274    0.2530
    Margin         9     0.258    0.3411         9     1.140    0.3545

   Species         6                             7
            Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
 Treatment
         1         3     1.899    0.3241         3     2.924    0.4602
         2         3     1.147    0.2156         2     5.118    0.7967
         3         3     2.610    0.5098         3     3.152    0.9620
    Margin         9     1.885    0.7104         8     3.558    1.1636

   Species         8                             9
            Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
 Treatment
         1         3     2.187    0.3491         3     1.488    0.2623
         2         3     2.526    0.1269         3     1.524    0.1789
         3         3     2.829    0.1563         3     1.513    0.0316
```

```
      Margin          9      2.514     0.3436           9      1.508     0.1603


     Species     Margin

             Nobservd       Mean        s.d.

   Treatment

           1         24      1.943     1.0560

           2         23      2.451     1.9841

           3         24      2.339     1.2080

      Margin         71      2.241     1.4599

Identifier Minimum Mean Maximum Values  Missing
        WU     83.10     416.4      1083        288           0
       WUE    0.3900     22.72     81.43        288           0


===== REML split-plot factorial analysis on WU for season Summer          =====


REML variance components analysis

Response variate:  WU
Fixed model:         Constant + Treatment + Species + Treatment.Species
Random model:       Rep + Rep.wplot + Rep.wplot.splot
Number of units:    72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WU

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                         Wald statistic    n.d.f.      F statistic    d.d.f.      F pr
Treatment                                    39.81          2            19.91       4.0   0.008
Species                                       7.40          7             1.06      42.0   0.408
Treatment.Species                            20.58         14             1.47      42.0   0.165


Table of predicted means for Treatment


Treatment        1          2          3
             400.0    400.0    474.0
```

```
Standard error: 12.15


Table of predicted means for Species


Species       1       2       4       5       6       7       8       9
          420.3   438.9   419.8   412.4   420.9   441.4   445.2   398.4


Standard error: 17.30

Table of predicted means for Treatment.Species


    Species        1        2        4        5        6        7        8        9
  Treatment
          1    389.1    454.1    403.5    389.9    371.6    437.3    381.7    372.8
          2    418.1    364.9    393.7    405.2    388.3    415.4    406.7    408.1
          3    453.8    497.6    462.3    442.2    502.9    471.6    547.0    414.4

Standard error: 28.02

========== Comparing means at the 5% level =========

Comparisons between Treatment means

        Mean
3      474.0  a
2      400.0  b
1      400.0  b

========= Summary of descriptive statistics ========

    Species           1                              2
            Nobservd       Mean       s.d.  Nobservd       Mean       s.d.
  Treatment
          1        3      389.1      21.77         3      454.1      52.38
          2        3      418.1      44.96         3      364.9      55.49
          3        3      453.8     106.04         3      497.6      51.85
     Margin        9      420.3      64.99         9      438.9      74.58

    Species           4                              5
            Nobservd       Mean       s.d.  Nobservd       Mean       s.d.
  Treatment
          1        3      403.5      67.15         3      389.9      31.18
          2        3      393.7      51.49         3      405.2      22.80
          3        3      462.3      49.90         3      442.2       8.98
     Margin        9      419.8      58.69         9      412.4      30.60

    Species           6                              7
            Nobservd       Mean       s.d.  Nobservd       Mean       s.d.
  Treatment
          1        3      371.6      29.10         3      437.3      35.08
          2        3      388.3      93.88         3      415.4      62.47
```

```
          3         3     502.9     33.89         3     471.6     44.67
     Margin         9     420.9     80.84         9     441.4     48.84

    Species         8                           9
             Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
  Treatment
          1         3     381.7     32.90         3     372.8     16.61
          2         3     406.7     10.33         3     408.1     55.08
          3         3     547.0     12.14         3     414.4     21.92
     Margin         9     445.2     79.31         9     398.4     36.38

    Species    Margin
             Nobservd      Mean      s.d.
  Treatment
          1        24     400.0     43.53
          2        24     400.0     48.72
          3        24     474.0     57.51
     Margin        72     424.7     60.72


===== REML split-plot factorial analysis on WUE for season Summer      =====

REML variance components analysis

Response variate:  WUE
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WUE

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                Wald statistic  n.d.f.   F statistic  d.d.f.    F pr
Treatment                           2.76       2          1.38     4.0   0.350
Species                           257.35       7         36.76    42.0  <0.001
Treatment.Species                  38.21      14          2.73    42.0   0.006

Table of predicted means for Treatment

Treatment      1       2       3
           27.21   33.28   29.87

Standard error: 3.330

Table of predicted means for Species

Species      1       2       4       5       6       7       8       9
         47.82   40.32    5.44   18.51   53.27   31.49   28.41   15.67
```

```
Standard error: 3.622

Table of predicted means for Treatment.Species

    Species        1        2        4        5        6        7        8        9
  Treatment
          1    34.05    37.54     4.62    16.09    62.27    18.90    26.91    17.28
          2    64.30    47.67     3.78    18.00    47.45    36.13    34.10    14.80
          3    45.12    35.75     7.92    21.43    50.10    39.43    24.23    14.94

Standard error: 5.530

========== Comparing means at the 5% level =========

Comparisons between Species means

       Mean
6     53.27  a
1     47.82  ab
2     40.32  bc
7     31.49  c
8     28.41  cd
5     18.51  de
9     15.67  ef
4      5.44  f

========= Summary of descriptive statistics ========

    Species         1                              2
             Nobservd      Mean      s.d.   Nobservd      Mean      s.d.
  Treatment
          1         3     34.05     3.417          3     37.54     4.671
          2         3     64.30    11.407          3     47.67    13.254
          3         3     45.12     5.681          3     35.75     9.048
     Margin         9     47.82    14.806          9     40.32    10.040

    Species         4                              5
             Nobservd      Mean      s.d.   Nobservd      Mean      s.d.
  Treatment
          1         3      4.62     3.557          3     16.09     8.984
          2         3      3.78     4.213          3     18.00    11.948
          3         3      7.92     6.103          3     21.43     8.164
     Margin         9      5.44     4.528          9     18.51     8.833

    Species         6                              7
             Nobservd      Mean      s.d.   Nobservd      Mean      s.d.
  Treatment
          1         3     62.27    16.865          3     18.90     6.350
          2         3     47.45    22.587          3     36.13    11.848
          3         3     50.10     5.849          3     39.43    16.759
     Margin         9     53.27    15.938          9     31.49    14.372

    Species         8                              9
             Nobservd      Mean      s.d.   Nobservd      Mean      s.d.
  Treatment
```

```
       1          3      26.91      2.801           3      17.28      4.483
       2          3      34.10      7.108           3      14.80      2.959
       3          3      24.23      3.598           3      14.94      0.319
  Margin          9      28.41      6.111           9      15.67      2.950

 Species     Margin
           Nobservd       Mean       s.d.
Treatment
       1         24      27.21     18.071
       2         24      33.28     21.694
       3         24      29.87     15.906
  Margin         72      30.12     18.613
```

A2 Statistical analysis of eight *Poaceae* species exposed to three water treatments during the autumn growth cycle from 1 Mar 2015 – 31 May 2015 (Cycle 2)

```
Autumn Harvest 3 all Nov2016.rtf

WUE of large biomass grasses: AUTUMN HARVEST 3 weight

     Identifier Minimum      Mean Maximum   Values   Missing

            FW     132.0      8205     28775        72         2

            DW     49.25      2066      6762        72         2

          DM %     17.07     26.42     37.31        72         2

           LAI   0.01010    0.8872     4.273        72         2    Skew

========== REML split-plot factorial analysis on fresh weight with all species ==========

REML variance components analysis

Response variate:  FW

Fixed model:          Constant + Treatment + Species + Treatment.Species

Random model:         Rep.wplot.splot

Number of units:      70 (2 units excluded due to zero weights or missing values)


Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                    Wald statistic    n.d.f.     F statistic    d.d.f.     F pr
```

```
Treatment                          21.75      2       10.87    46.0 <0.001

Species                           114.67      7       16.38    46.0 <0.001

Treatment.Species                  17.70     14        1.26    46.0  0.266

Table of predicted means for Treatment

Treatment       1       2       3

             5123    9971   10053

Standard errors

Treatment       1       2       3

            818.0   843.1   843.1

Standard errors

Average:                 834.7

Maximum:                 843.1

Minimum:                 818.0

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

          11360   10513     843    8041    8309   18797    2960    6238

Standard errors

Species       1       2       4       5       6       7       8       9

           1336    1336    1336    1336    1336    1443    1336    1443

Standard errors

Average:                 1362.

Maximum:                 1443.

Minimum:                 1336.

Table of predicted means for Treatment.Species

    Species       1       2       4       5       6       7       8       9

  Treatment

          1    6798    6397     226    5557    5253   14333    1177    1247

          2   18592   14039     597    8451    8552   18911    3463    7163
```

```
          3    8690  11102    1706  10114  11122  23145    4241  10304

Standard errors

   Species       1       2       4       5       6       7       8       9

Treatment

         1    2314    2314    2314    2314    2314    2314    2314    2314

         2    2314    2314    2314    2314    2314    2833    2314    2314

         3    2314    2314    2314    2314    2314    2314    2314    2833

Standard errors

Average:               2357.

Maximum:               2833.

Minimum:               2314.

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

          Mean

3        10053   a

2         9971   a

1         5123   b

Comparisons between Species means

          Mean

7        18797   a

1        11360   b

2        10513   b

6         8309   bc

5         8041   bc

9         6238   bcd

8         2960   cd

4          843   d

   ============= Summary of data for SDs ============
```

Species	1			2		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	6798	1860	3	6397	3176
2	3	18592	10974	3	14039	10416
3	3	8690	1523	3	11102	4003
Margin	9	11360	7851	9	10513	6693
Species	4			5		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	226	96	3	5557	2548
2	3	597	492	3	8451	1030
3	3	1706	1252	3	10114	803
Margin	9	843	948	9	8041	2457
Species	6			7		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	5253	3700	3	14333	3533
2	3	8552	826	2	18911	10096
3	3	11122	556	3	23145	1630
Margin	9	8309	3188	8	18782	5961

Species	8			9		
	Nobservd	Mean	s.d.	Nobservd	Mean	s.d.
Treatment						
1	3	1177	333	3	1247	1377
2	3	3463	2920	3	7163	2193

```
         3         3      4241       906           2     10304      1762

    Margin         9      2960      2066           8      5730      4223

   Species    Margin

            Nobservd      Mean      s.d.

 Treatment

         1        24      5123      4794

         2        23      9582      8109

         3        23     10042      6356

    Margin        70      8205      6824

========== REML split-plot factorial analysis on dry weight with all species ==========

REML variance components analysis

Response variate:  DW

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep.wplot.splot

Number of units:   70 (2 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                  Wald statistic   n.d.f.    F statistic   d.d.f.    F pr

Treatment                            19.72        2           9.86     46.0 <0.001

Species                             148.64        7          21.23     46.0 <0.001

Treatment.Species                    18.48       14           1.32     46.0  0.233

Table of predicted means for Treatment

Treatment       1       2       3

             1432    2379    2493

Standard errors

Treatment       1       2       3
```

```
          178.9   184.4   184.4

Standard errors

Average:                 182.5

Maximum:                 184.4

Minimum:                 178.9

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

           3110    2017     257    2580    2380    4459     590    1417

Standard errors

Species       1       2       4       5       6       7       8       9

          292.1   292.1   292.1   292.1   292.1   315.5   292.1   315.5

Standard errors

Average:                 297.9

Maximum:                 315.5

Minimum:                 292.1

Table of predicted means for Treatment.Species

     Species        1       2       4       5       6       7       8       9

   Treatment

            1    2127    1486      84    1878    1664    3679     273     268

            2    4654    2620     173    2677    2317    4303     672    1614

            3    2548    1946     514    3185    3159    5396     825    2368



Standard errors

   Species         1       2       4       5       6       7       8       9

 Treatment

          1    505.9   505.9   505.9   505.9   505.9   505.9   505.9   505.9

          2    505.9   505.9   505.9   505.9   505.9   619.6   505.9   505.9
```

```
          3   505.9   505.9   505.9   505.9   505.9   505.9   505.9   619.6

Standard errors

Average:                515.4

Maximum:                619.6

Minimum:                505.9

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

        Mean

3        2493   a

2        2379   a

1        1432   b

Comparisons between Species means

        Mean

7        4459   a

1        3110   b

5        2580   bc

6        2380   bc

2        2017   bc

9        1417   cd

8         590   d

4         257   d

   ============= Summary of data for SDs ============


   Species          1                           2

            Nobservd     Mean      s.d.  Nobservd     Mean      s.d.

 Treatment

         1          3     2127     533.1         3     1486     728.1

         2          3     4654    2393.1         3     2620    1860.2
```

```
           3         3      2548     410.0         3      1946     675.0

      Margin         9      3110    1708.6         9      2017    1164.3

     Species         4                             5

              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.

   Treatment

           1         3        84      34.6         3      1878     858.2

           2         3       173     132.4         3      2677     331.5

           3         3       514     370.9         3      3185     190.4

      Margin         9       257     279.0         9      2580     739.1

     Species         6                             7

              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.

   Treatment

           1         3      1664    1165.6         3      3679     970.7

           2         3      2317     221.8         2      4303    2201.2

           3         3      3159     367.5         3      5396     333.8

      Margin         9      2380     898.6         8      4479    1279.4

     Species         8                             9

              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.

   Treatment

           1         3       273      88.9         3       268     273.0

           2         3       672     584.9         3      1614     546.2

           3         3       825     256.9         2      2368     611.0

      Margin         9       590     406.1         8      1298     992.2

     Species    Margin

              Nobservd      Mean      s.d.

   Treatment

           1        24      1432    1298.6

           2        23      2295    1823.8
```

```
           3         23       2498     1532.6
      Margin         70       2066     1609.6
========== REML split-plot factorial analysis on dry matter % with all species ==========
REML variance components analysis
Response variate:  DM %
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep.wplot.splot
Number of units:   70 (2 units excluded due to zero weights or missing values)
Rep.wplot.splot used as residual term
Sparse algorithm with AI optimisation
Tests for fixed effects
Sequentially adding terms to fixed model
Fixed term                 Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                           54.00        2         27.00     46.0  <0.001
Species                            486.23        7         69.46     46.0  <0.001
Treatment.Species                   18.42       14          1.32     46.0   0.235
Table of predicted means for Treatment
Treatment       1       2       3
            28.63   24.84   25.47
Standard errors
Treatment       1       2       3
             0.38    0.39    0.39

Standard errors
 Average: 0.3852
Maximum:              0.3891
Minimum:              0.3774
Table of predicted means for Species
```

```
Species        1        2        4        5        6        7        8        9

           29.10    20.01    32.80    32.34    29.10    23.95    20.48    22.76

Standard errors

Species        1        2        4        5        6        7        8        9

            0.62     0.62     0.62     0.62     0.62     0.67     0.62     0.67

Standard errors

Average:                0.6287

Maximum:                0.6658

Minimum:                0.6164

Table of predicted means for Treatment.Species

     Species        1        2        4        5        6        7        8        9

   Treatment

           1    31.41    23.36    37.07    33.78    31.75    25.59    23.02    23.09

           2    26.47    19.08    29.89    31.70    27.11    22.91    19.20    22.39

           3    29.41    17.60    31.45    31.53    28.43    23.36    19.21    22.81

Standard errors

   Species        1        2        4        5        6        7        8        9

 Treatment

         1     1.07     1.07     1.07     1.07     1.07     1.07     1.07     1.07

         2     1.07     1.07     1.07     1.07     1.07     1.31     1.07     1.07

         3     1.07     1.07     1.07     1.07     1.07     1.07     1.07     1.31


 Standard errors

Average:                1.088

Maximum:                1.308

Minimum:                1.068

   ========= Comparing means at the 5% level ========

Comparisons between Treatment means
```

```
          Mean
1         28.63   a
3         25.47   b
2         24.84   b
Comparisons between Species means
          Mean
4         32.80   a
5         32.34   a
1         29.10   b
6         29.10   b
7         23.95   c
9         22.76   cd
8         20.48   d
2         20.01   d
   ============= Summary of data for SDs ============
     Species            1                              2
              Nobservd       Mean        s.d.  Nobservd       Mean        s.d.
   Treatment
           1            3       31.41       0.778           3       23.36       1.331
           2            3       26.47       2.674           3       19.08       0.858
           3            3       29.41       1.889           3       17.60       0.647
      Margin            9       29.10       2.731           9       20.01       2.725


     Species            4                              5
              Nobservd       Mean        s.d.  Nobservd       Mean        s.d.
   Treatment
           1            3       37.07       0.333           3       33.78       1.432
```

```
        2          3      29.89      4.048          3      31.70      1.672
        3          3      31.45      3.095          3      31.53      1.193
   Margin          9      32.80      4.147          9      32.34      1.659
  Species          6                                7
            Nobservd       Mean       s.d.   Nobservd       Mean       s.d.
Treatment
        1          3      31.75      0.177          3      25.59      1.846
        2          3      27.11      1.128          2      22.91      0.592
        3          3      28.43      3.384          3      23.36      1.587
   Margin          9      29.10      2.736          8      24.08      1.825
  Species          8                                9
            Nobservd       Mean       s.d.   Nobservd       Mean       s.d.
Treatment
        1          3      23.02      1.254          3      23.09      2.284
        2          3      19.20      0.649          3      22.39      0.710
        3          3      19.21      1.850          2      22.81      2.028
   Margin          9      20.48      2.233          8      22.76      1.526
  Species     Margin
            Nobservd       Mean       s.d.
Treatment
        1         24      28.63      5.409
        2         23      24.93      4.861
        3         23      25.59      5.600
   Margin         70      26.42      5.474
========== REML split-plot factorial analysis on LAI with all species ==========
REML variance components analysis
Response variate:  LAI
Fixed model:           Constant + Treatment + Species + Treatment.Species
```

```
Random model:     Rep.wplot.splot

Number of units:  70 (2 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                  Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                            15.48        2          7.74     46.0   0.001

Species                             228.94        7         32.71     46.0  <0.001

Treatment.Species                    21.16       14          1.51     46.0   0.145

Table of predicted means for Treatment

Treatment       1       2       3

           0.6000  1.0207  1.1092

Standard errors

Treatment       1       2       3

           0.0945  0.0974  0.0974

Standard errors

Average:            0.09647

Maximum:            0.09744

Minimum:            0.09453

Table of predicted means for Species



Species       1       2       4       5       6       7       8       9

         1.3168  1.0857  0.0825  0.3728  0.7568  2.8895  0.2541  0.5217

Standard errors

Species       1       2       4       5       6       7       8       9

         0.1544  0.1544  0.1544  0.1544  0.1544  0.1667  0.1544  0.1667
```

```
Standard errors

Average:            0.1575

Maximum:            0.1667

Minimum:            0.1544

Table of predicted means for Treatment.Species

    Species      1      2      4      5      6      7      8      9

  Treatment

            1 0.6867 0.7699 0.0204 0.2176 0.4108 2.4773 0.1136 0.1037

            2 2.1031 1.4333 0.0484 0.3841 0.7619 2.5289 0.3326 0.5733

            3 1.1607 1.0538 0.1786 0.5168 1.0976 3.6623 0.3161 0.8880

Standard errors

  Species      1      2      4      5      6      7      8      9

Treatment

          1 0.2674 0.2674 0.2674 0.2674 0.2674 0.2674 0.2674 0.2674

          2 0.2674 0.2674 0.2674 0.2674 0.2674 0.3275 0.2674 0.2674

          3 0.2674 0.2674 0.2674 0.2674 0.2674 0.2674 0.2674 0.3275

Standard errors

Average:            0.2724

Maximum:            0.3275

Minimum:            0.2674

   ========== Comparing means at the 5% level =========



Comparisons between Treatment means

       Mean

3     1.1092  a

2     1.0207  a

1     0.6000  b
```

```
Comparisons between Species means

          Mean

7       2.8895   a

1       1.3168   b

2       1.0857   bc

6       0.7568   bcd

9       0.5217   cde

5       0.3728   de

8       0.2541   de

4       0.0825   e

   ============= Summary of data for SDs ============

    Species           1                           2

          Nobservd      Mean       s.d.  Nobservd      Mean       s.d.

  Treatment

          1        3    0.6867     0.3531        3    0.7699     0.3708

          2        3    2.1031     1.0528        3    1.4333     1.1387

          3        3    1.1607     0.1845        3    1.0538     0.4703

     Margin        9    1.3168     0.8406        9    1.0857     0.7049

    Species        4                             5

          Nobservd      Mean       s.d.  Nobservd      Mean       s.d.

  Treatment

          1        3    0.0204     0.0109        3    0.2176     0.0977

          2        3    0.0484     0.0353        3    0.3841     0.0492

          3        3    0.1786     0.1375        3    0.5168     0.0825

     Margin        9    0.0825     0.1021        9    0.3728     0.1468

    Species        6                             7

          Nobservd      Mean       s.d.  Nobservd      Mean       s.d.

  Treatment
```

```
            1          3     0.4108     0.3032            3     2.4773     0.9299

            2          3     0.7619     0.0580            2     2.5289     1.0179

            3          3     1.0976     0.3126            3     3.6623     0.5308

       Margin          9     0.7568     0.3697            8     2.9346     0.9161

      Species          8                                  9

                Nobservd       Mean       s.d.  Nobservd       Mean       s.d.

    Treatment

            1          3     0.1136     0.0286            3     0.1037     0.1019

            2          3     0.3326     0.2472            3     0.5733     0.1852

            3          3     0.3161     0.0747            2     0.8880     0.0557

       Margin          9     0.2541     0.1674            8     0.4759     0.3538

      Species     Margin

                Nobservd       Mean       s.d.

    Treatment

            1         24     0.6000     0.8383

            2         23     0.9551     0.9724

            3         23     1.1189     1.0984

       Margin         70     0.8872     0.9841

===== REML split-plot factorial analysis on WU for season Autumn =====

REML variance components analysis

Response variate:  WU
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WU

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                   Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                            612.99        2        306.49      4.0 <0.001
```

```
Species                          65.80       7          9.40     42.0 <0.001
Treatment.Species                33.55      14          2.40     42.0   0.015

Table of predicted means for Treatment

Treatment      1         2         3
            99.3   162.5   212.6

Standard error: 4.224

Table of predicted means for Species

Species      1         2         4         5         6         7         8         9
         144.5   153.9   170.0   166.8   167.2   150.0   139.5   173.2

Standard error: 5.101

Table of predicted means for Treatment.Species

     Species        1         2         4         5         6         7         8         9
   Treatment
           1    96.0     98.2   101.7     98.3   104.9     96.2     92.0   107.5
           2   130.7   161.6   179.1   179.7   179.4   138.7   144.3   186.7
           3   206.9   202.0   229.3   222.5   217.4   215.2   182.2   225.4

Standard error: 7.962

========== Comparing means at the 5% level =========
Comparisons between Treatment means

        Mean
3       212.6   a
2       162.5   b
1         99.3  c

Comparisons between Species means

        Mean
9       173.2   a
4       170.0   ab
6       167.2   abc
5       166.8   abc
2       153.9   bcd
7       150.0   cd
1       144.5   d
8       139.5   d

========= Summary of descriptive statistics ========

     Species           1                                    2
            Nobservd       Mean          s.d.  Nobservd       Mean          s.d.
   Treatment
           1           3       96.0        1.28           3       98.2        4.61
           2           3     130.7        4.26           3     161.6        5.13
           3           3     206.9      10.83           3     202.0      26.08
```

```
      Margin         9     144.5     49.48         9     153.9     47.30

     Species         4                             5
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3     101.7      7.98         3      98.3      1.89
           2         3     179.1     13.57         3     179.7     17.89
           3         3     229.3     16.08         3     222.5     31.37
      Margin         9     170.0     56.82         9     166.8     57.57

     Species         6                             7
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3     104.9      1.93         3      96.2      8.20
           2         3     179.4     22.33         3     138.7      9.72
           3         3     217.4      3.78         3     215.2      9.65
      Margin         9     167.2     50.87         9     150.0     52.83

     Species         8                             9
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3      92.0      7.76         3     107.5      8.97
           2         3     144.3     24.85         3     186.7     11.42
           3         3     182.2     14.21         3     225.4      4.80
      Margin         9     139.5     41.93         9     173.2     52.61

     Species    Margin
              Nobservd      Mean      s.d.
   Treatment
           1        24      99.3      7.05
           2        24     162.5     24.53
           3        24     212.6     20.50
      Margin        72     158.2     50.26


===== REML split-plot factorial analysis on WUE for season Autumn      =====

REML variance components analysis

Response variate:  WUE
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WUE

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                           4.39        2          2.20      6.0   0.192
```

```
Species                           128.70      7        18.39    42.0 <0.001
Treatment.Species                  25.84     14         1.85    42.0   0.063

Table of predicted means for Treatment

Treatment      1        2        3
           14.65    15.33    11.01

Standard error: 1.568

Table of predicted means for Species

Species      1        2        4        5        6        7        8        9
         23.34    13.59     1.36    16.29    14.45    29.68     4.04     6.57

Standard error: 2.291

Table of predicted means for Treatment.Species

     Species        1        2        4        5        6        7        8        9
   Treatment
           1    22.19    14.96     0.84    19.21    15.77    38.66     2.93     2.65
           2    35.41    16.18     0.97    15.11    13.02    28.67     4.58     8.72
           3    12.40     9.64     2.28    14.56    14.55    21.71     4.60     8.34

Standard error: 3.969

========== Comparing means at the 5% level =========

Comparisons between Species means

        Mean
7      29.68  a
1      23.34  ab
5      16.29  bc
6      14.45  bc
2      13.59  bcd
9       6.57  cde
8       4.04  de
4       1.36  e

========= Summary of descriptive statistics ========

     Species           1                          2
                Nobservd     Mean      s.d. Nobservd     Mean      s.d.
   Treatment
           1           3    22.19     5.727        3    14.96     6.644
           2           3    35.41    17.567        3    16.18    11.611
           3           3    12.40     2.561        3     9.64     2.919
      Margin           9    23.34    13.675        9    13.59     7.479


     Species           4                          5
                Nobservd     Mean      s.d. Nobservd     Mean      s.d.
   Treatment
```

```
            1         3      0.84     0.400         3     19.21     9.162
            2         3      0.97     0.731         3     15.11     3.158
            3         3      2.28     1.645         3     14.56     2.678
       Margin         9      1.36     1.153         9     16.29     5.487

      Species         6                             7
               Nobservd      Mean      s.d. Nobservd      Mean      s.d.
    Treatment
            1         3     15.77    10.998         3     38.66    12.095
            2         3     13.02     1.688         3     28.67    10.010
            3         3     14.55     1.919         3     21.71     8.392
       Margin         9     14.45     5.770         9     29.68    11.561

      Species         8                             9
               Nobservd      Mean      s.d. Nobservd      Mean      s.d.
    Treatment
            1         3      2.93     0.770         3      2.65     2.895
            2         3      4.58     3.477         3      8.72     3.202
            3         3      4.60     1.717         3      8.34     3.944
       Margin         9      4.04     2.143         9      6.57     4.150

      Species    Margin
               Nobservd      Mean      s.d.
    Treatment
            1        24     14.65    13.604
            2        24     15.33    13.216
            3        24     11.01     6.720
       Margin        72     13.67    11.611
```

A3 Statistical analysis of eight *Poaceae* species exposed to three water treatments during the spring growth cycle from 1 Sep 2015 – 30 Nov 2015 (Cycle 3)

```
Spring Harvest 3 all Nov2016.rtf

WUE of large biomass grasses: SPRING HARVEST 3 weight




     Identifier Minimum     Mean Maximum   Values  Missing

             FW   850.0    22943   71925       72        2

             DW   283.3     5633   18938       72        2

           DM %   9.524    24.56   44.44       72        2

            LAI 0.02556    1.564   6.574       72        2    Skew

========== REML split-plot factorial analysis on fresh weight with all species ==========

REML variance components analysis
```

```
Response variate:  FW

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep.wplot.splot

Number of units:   70 (2 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                  Wald statistic   n.d.f.    F statistic   d.d.f.    F pr

Treatment                            88.56        2          44.28     46.0 <0.001

Species                             127.66        7          18.24     46.0 <0.001

Treatment.Species                    47.15       14           3.37     46.0 <0.001

Table of predicted means for Treatment

Treatment       1        2        3

            12095    25158    31770

Standard errors

Treatment       1        2        3

             1516     1563     1563

Standard errors

Average:               1547.

Maximum:               1563.

Minimum:               1516.

Table of predicted means for Species

Species       1        2        4        5        6        7        8        9

          19894    38192     9558    37912    26494    18478    20778    12756

Standard errors

Species       1        2        4        5        6        7        8        9

           2475     2475     2475     2475     2475     2674     2475     2674
```

```
Standard errors

Average:                 2525.

Maximum:                 2674.

Minimum:                 2475.

Table of predicted means for Treatment.Species

     Species        1       2       4       5       6       7       8       9

   Treatment

           1    10083   18433    3475   16719   17917    9150   11692    9292

           2    19533   42308   16358   46883   28708   28100   12875    6500

           3    30067   53833    8842   50133   32858   18183   37767   22475

Standard errors

   Species        1       2       4       5       6       7       8       9

 Treatment

         1     4288    4288    4288    4288    4288    4288    4288    4288

         2     4288    4288    4288    4288    4288    5251    4288    4288

         3     4288    4288    4288    4288    4288    4288    4288    5251

Standard errors

Average:                 4368.

Maximum:                 5251.

Minimum:                 4288.

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

         Mean

3       31770   a

2       25158   b

1       12095   c

Comparisons between Species means

         Mean
```

2	38192	a
5	37912	a
6	26494	b
8	20778	bc
1	19894	bcd
7	18478	bcd
9	12756	cd
4	9558	d

Comparisons between Treatment.Species means

Variances vary and decisions regarding group membership are inconsistent, so there may be gaps in the lines or letters linking means in identical groups.

	Mean	
3 2	53833	a
3 5	50133	ab
2 5	46883	abc
2 2	42308	abcd
3 8	37767	abcde
3 6	32858	abcdef
3 1	30067	bcdefg
2 6	28708	bcdefgh
2 7	28100	abcdefghi
3 9	22475	cdefghi
2 1	19533	defghi
1 2	18433	efghi
3 7	18183	efghi
1 6	17917	efghi
1 5	16719	efghi
2 4	16358	efghi
2 8	12875	fghi

```
1 8       11692   fghi

1 1       10083   fghi

1 9        9292   ghi

1 7        9150   ghi

3 4        8842   ghi

2 9        6500   hi

1 4        3475

   ============= Summary of data for SDs ============

      Species          1                        2

              Nobservd       Mean       s.d. Nobservd       Mean       s.d.

    Treatment

            1          3      10083       4028        3      18433       5338

            2          3      19533       3955        3      42308       7349

            3          3      30067       3426        3      53833      15669

       Margin          9      19894       9265        9      38192      18069

      Species          4                        5

              Nobservd       Mean       s.d. Nobservd       Mean       s.d.

    Treatment

            1          3       3475       2625        3      16719       3249

            2          3      16358       9805        3      46883      12331

            3          3       8842       5552        3      50133      13798

       Margin          9       9558       8055        9      37912      18517

      Species          6                        7

              Nobservd       Mean       s.d. Nobservd       Mean       s.d.

    Treatment

            1          3      17917       8247        3       9150        513

            2          3      28708       8615        2      28100      10571

            3          3      32858        726        3      18183       2517
```

```
   Margin           9     26494      8961           8     17275      8943
  Species           8                               9
             Nobservd      Mean      s.d. Nobservd      Mean      s.d.
Treatment
        1           3     11692      4525           3      9292      2843
        2           3     12875      6191           3      6500      3416
        3           3     37767      8540           2     22475     10324
   Margin           9     20778     13984           8     11541      8251
  Species      Margin
             Nobservd      Mean      s.d.
Treatment
        1          24     12095      6219
        2          23     25030     15334
        3          23     32174     16672
   Margin          70     22943     15725
========== REML split-plot factorial analysis on dry weight with all species ==========
REML variance components analysis
Response variate:  DW
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep.wplot.splot
Number of units:   70 (2 units excluded due to zero weights or missing values)
Rep.wplot.splot used as residual term
Sparse algorithm with AI optimisation
Tests for fixed effects
Sequentially adding terms to fixed model
Fixed term                Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                          81.30        2         40.65     46.0 <0.001
Species                           212.20        7         30.31     46.0 <0.001
```

```
Treatment.Species                 52.76      14          3.77     46.0 <0.001

Table of predicted means for Treatment

Treatment       1       2       3

             3006    6444    7411

Standard errors

Treatment       1       2       3

            370.3   381.7   381.7

Standard errors

Average:                377.9

Maximum:                381.7

Minimum:                370.3

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

           5790    7812    3164   12502    6155    4393    3038    2106

Standard errors

Species       1       2       4       5       6       7       8       9

          604.7   604.7   604.7   604.7   604.7   653.1   604.7   653.1

Standard errors

Average:                616.8

Maximum:                653.1

Minimum:                604.7

Table of predicted means for Treatment.Species

    Species       1       2       4       5       6       7       8       9

  Treatment

          1    2605    4328    1220    5670    4557    2154    1673    1839

          2    5705    8996    4773   15673    6608    6928    1823    1043

          3    9061   10112    3500   16162    7301    4097    5617    3435

Standard errors
```

```
  Species        1        2        4        5        6        7        8        9
Treatment
        1     1047     1047     1047     1047     1047     1047     1047     1047
        2     1047     1047     1047     1047     1047     1283     1047     1047
        3     1047     1047     1047     1047     1047     1047     1047     1283
Standard errors
Average:                1067.
Maximum:                1283.
Minimum:                1047.
   ========== Comparing means at the 5% level =========
Comparisons between Treatment means
        Mean
3       7411  a
2       6444  a
1       3006  b
Comparisons between Species means
 Mean
5      12502  a
2       7812  b
6       6155  bc
1       5790  bcd
7       4393  cde
4       3164  de
8       3038  e
9       2106  e
Comparisons between Treatment.Species means
          Mean
3 5      16162  a
```

```
2 5      15673  ab

3 2      10112  bc

3 1       9061  cd

2 2       8996  cd

3 6       7301  cde

2 7       6928  cdef

2 6       6608  cdef

2 1       5705  cdef

1 5       5670  cdef

3 8       5617  cdef

2 4       4773  cdef

1 6       4557  cdef

1 2       4328  def

3 7       4097  def

3 4       3500  def

3 9       3435  def

1 1       2605  ef

1 7       2154  ef

1 9       1839  ef

2 8       1823  ef

1 8       1673  ef

1 4       1220  f

2 9       1043  f

   ============ Summary of data for SDs ============

     Species          1                         2

            Nobservd     Mean      s.d.  Nobservd     Mean      s.d.

   Treatment

           1        3     2605     1096         3     4328      1117
```

```
           2         3      5705      1398         3      8996       914
           3         3      9061       736         3     10112      2729
      Margin         9      5790      2957         9      7812      3073
     Species         4                             5
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3      1220       969         3      5670       627
           2         3      4773      3492         3     15673      3789
           3         3      3500      2125         3     16162      3421
      Margin         9      3164      2616         9     12502      5737
     Species         6                             7
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3      4557      1535         3      2154       135
           2         3      6608      1717         2      6928      2889
           3         3      7301      1336         3      4097       782
      Margin         9      6155      1816         8      4076      2298
     Species         8                             9
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3      1673       427         3      1839       680
           2         3      1823      1417         3      1043       600
           3         3      5617      1667         2      3435      1158
      Margin         9      3038      2234         8      1940      1189
     Species    Margin
              Nobservd      Mean      s.d.
   Treatment
           1        24      3006      1732
```

```
           2       23      6423      4838

           3       23      7583      4469

      Margin       70      5633      4325

========== REML split-plot factorial analysis on dry matter % with all species ==========

REML variance components analysis

Response variate:  DM %

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep.wplot.splot

Number of units:   70 (2 units excluded due to zero weights or missing values)

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                  Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                             7.93        2          3.97     46.0   0.026

Species                             532.37        7         76.05     46.0  <0.001

Treatment.Species                    58.20       14          4.16     46.0  <0.001

Table of predicted means for Treatment

Treatment       1       2       3

            25.26   23.45   24.60


Standard errors

Treatment       1       2       3

             0.49    0.51    0.51

Standard errors

Average:             0.5047

Maximum:             0.5098

Minimum:             0.4945
```

```
Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

          28.31   21.34   33.79   33.50   23.89   23.47   14.17   17.03

Standard errors

Species       1       2       4       5       6       7       8       9

           0.81    0.81    0.81    0.81    0.81    0.87    0.81    0.87

Standard errors

Average:              0.8238

Maximum:              0.8723

Minimum:              0.8076

Table of predicted means for Treatment.Species

    Species        1       2       4       5       6       7       8       9

  Treatment

              1   25.62   23.69   34.34   34.26   26.31   23.53   14.85   19.52

              2   29.04   21.47   27.12   33.59   23.19   24.45   12.92   15.82

              3   30.28   18.86   39.90   32.65   22.17   22.43   14.75   15.76

Standard errors

  Species        1       2       4       5       6       7       8       9

Treatment

            1   1.40    1.40    1.40    1.40    1.40    1.40    1.40    1.40

            2   1.40    1.40    1.40    1.40    1.40    1.71    1.40    1.40

            3   1.40    1.40    1.40    1.40    1.40    1.40    1.40    1.71

Standard errors

Average:               1.425

Maximum:               1.713

Minimum:               1.399

   ========= Comparing means at the 5% level ========

Comparisons between Treatment means
```

	Mean	
1	25.26	a
3	24.60	ab
2	23.45	b

Comparisons between Species means

	Mean	
4	33.79	a
5	33.50	a
1	28.31	b
6	23.89	c
7	23.47	c
2	21.34	c
9	17.03	d
8	14.17	d

Comparisons between Treatment.Species means

Variances vary and decisions regarding group membership are inconsistent, so there may be gaps in the lines or letters linking means in identical groups.

	Mean	
3 4	39.90	a
1 4	34.34	ab
1 5	34.26	ab
2 5	33.59	abc
3 5	32.65	abcd
3 1	30.28	bcde
2 1	29.04	bcdef
2 4	27.12	bcdef
1 6	26.31	cdefg
1 1	25.62	defg
2 7	24.45	defgh

```
1 2        23.69  efgh
1 7        23.53  efgh
2 6        23.19  efghi
3 7        22.43  fghij
3 6        22.17  fghijk
2 2        21.47  fghijk
1 9        19.52  ghijkl
3 2        18.86  ghijkl
2 9        15.82  ijkl
3 9        15.76  hijkl
1 8        14.85  jkl
3 8        14.75  kl
2 8        12.92  l
  ============= Summary of data for SDs ============
     Species           1                          2
                Nobservd      Mean       s.d.  Nobservd      Mean       s.d.
   Treatment
           1           3     25.62      1.445         3     23.69      1.608
           2           3     29.04      1.263         3     21.47      1.905
           3           3     30.28      2.466         3     18.86      0.760
      Margin           9     28.31      2.608         9     21.34      2.465
     Species           4                          5
                Nobservd      Mean       s.d.  Nobservd      Mean       s.d.
   Treatment
           1           3     34.34      1.750         3     34.26      2.645
           2           3     27.12      5.671         3     33.59      1.538
           3           3     39.90      4.134         3     32.65      2.382
      Margin           9     33.79      6.623         9     33.50      2.061
```

```
     Species         6                             7
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3     26.31     2.872         3     23.53     0.277
           2         3     23.19     1.030         2     24.45     1.084
           3         3     22.17     3.622         3     22.43     1.195
      Margin         9     23.89     3.015         8     23.35     1.150
     Species         8                             9
              Nobservd      Mean      s.d.  Nobservd      Mean      s.d.
   Treatment
           1         3     14.85     2.134         3     19.52     1.477
           2         3     12.92     3.940         3     15.82     0.834
           3         3     14.75     1.199         2     15.76     2.090
      Margin         9     14.17     2.502         8     17.19     2.270
     Species    Margin
              Nobservd      Mean      s.d.
   Treatment
           1        24     25.26     6.574
           2        23     23.41     7.004
           3        23     24.98     8.695
      Margin        70     24.56     7.405
========== REML split-plot factorial analysis on LAI with all species ==========
REML variance components analysis
Response variate:  LAI
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep.wplot.splot
Number of units:   70 (2 units excluded due to zero weights or missing values)
Rep.wplot.splot used as residual term
```

```
Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                     Wald statistic   n.d.f.    F statistic   d.d.f.    F pr

Treatment                               78.42        2          39.21     46.0 <0.001

Species                                222.66        7          31.81     46.0 <0.001

Treatment.Species                       95.01       14           6.79     46.0 <0.001

Table of predicted means for Treatment

Treatment        1        2        3

             0.916    1.585    2.326

Standard errors

Treatment        1        2        3

             0.108    0.111    0.111

Standard errors

Average:                0.1101

Maximum:                0.1112

Minimum:                0.1079

Table of predicted means for Species

Species        1        2        4        5        6        7        8        9

           1.383    2.164    0.238    1.326    0.304    2.227    3.210    2.019


Standard errors

Species        1        2        4        5        6        7        8        9

           0.176    0.176    0.176    0.176    0.176    0.190    0.176    0.190

Standard errors

Average:                0.1797

Maximum:                0.1903

Minimum:                0.1762
```

```
Table of predicted means for Treatment.Species

      Species      1      2      4      5      6      7      8      9

    Treatment

            1  0.699  1.167  0.128  0.387  0.306  1.094  1.987  1.564

            2  1.344  2.464  0.349  2.018  0.325  2.974  2.068  1.136

            3  2.107  2.860  0.236  1.574  0.281  2.614  5.575  3.358

Standard errors

    Species      1      2      4      5      6      7      8      9

 Treatment

          1  0.305  0.305  0.305  0.305  0.305  0.305  0.305  0.305

          2  0.305  0.305  0.305  0.305  0.305  0.374  0.305  0.305

          3  0.305  0.305  0.305  0.305  0.305  0.305  0.305  0.374

Standard errors

Average:             0.3109

Maximum:             0.3738

Minimum:             0.3052

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

          Mean

3         2.326  a

2         1.585  b

1         0.916  c

Comparisons between Species means

          Mean

8         3.210  a

7         2.227  b

2         2.164  b

9         2.019  bc
```

1	1.383	c
5	1.326	c
6	0.304	d
4	0.238	d

Comparisons between Treatment.Species means

	Mean	
3 8	5.575	a
3 9	3.358	b
2 7	2.974	bc
3 2	2.860	bcd
3 7	2.614	bcde
2 2	2.464	bcde
3 1	2.107	bcdef
2 8	2.068	bcdef
2 5	2.018	bcdefg
1 8	1.987	bcdefgh
3 5	1.574	bcdefghi
1 9	1.564	bcdefghi
2 1	1.344	cdefghi
1 2	1.167	cefghi
2 9	1.136	cefghi
1 7	1.094	efghi
1 1	0.699	fghi
1 5	0.387	ghi
2 4	0.349	hi
2 6	0.325	i
1 6	0.306	i
3 6	0.281	i

```
3 4        0.236   i
1 4        0.128   i
   ============ Summary of data for SDs ===========
     Species           1                         2
               Nobservd       Mean       s.d. Nobservd       Mean       s.d.
   Treatment
           1           3     0.699     0.2664          3     1.167     0.2410
           2           3     1.344     0.2357          3     2.464     0.1725
           3           3     2.107     0.9488          3     2.860     0.8291
      Margin           9     1.383     0.7933          9     2.164     0.8842
     Species           4                         5
               Nobservd       Mean       s.d. Nobservd       Mean       s.d.
   Treatment
           1           3     0.128     0.1127          3     0.387     0.0827
           2           3     0.349     0.2131          3     2.018     0.7617
           3           3     0.236     0.0965          3     1.574     0.2720
      Margin           9     0.238     0.1613          9     1.326     0.8357
     Species           6                         7
               Nobservd       Mean       s.d. Nobservd       Mean       s.d.
   Treatment
           1           3     0.306     0.3007          3     1.094     0.0844
           2           3     0.325     0.0976          2     2.974     1.0105
           3           3     0.281     0.1451          3     2.614     0.3464
      Margin           9     0.304     0.1750          8     2.134     0.9726
     Species           8                         9
               Nobservd       Mean       s.d. Nobservd       Mean       s.d.
   Treatment
           1           3     1.987     0.6835          3     1.564     0.5571
```

```
         2         3     2.068    1.0162          3     1.136    0.5321

         3         3     5.575    0.9471          2     3.358    0.5429

    Margin         9     3.210    1.9356          8     1.852    1.0560

   Species    Margin

            Nobservd      Mean      s.d.

 Treatment

         1        24     0.916    0.6914

         2        23     1.524    1.0129

         3        23     2.281    1.7588

    Margin        70     1.564    1.3380

===== REML split-plot factorial analysis on WU for season Spring =====

REML variance components analysis

Response variate:  WU
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WU

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                 Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                           73.82        2         36.91      6.0  <0.001
Species                              9.31        7          1.33     42.0   0.260
Treatment.Species                   18.37       14          1.31     42.0   0.241

Table of predicted means for Treatment

Treatment       1       2       3
            145.6   292.6   335.4

Standard error: 16.39

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9
          256.1   267.0   239.9   269.5   246.1   236.1   263.3   284.8
```

```
Standard error: 16.40

Table of predicted means for Treatment.Species

     Species       1       2       4       5       6       7       8       9
   Treatment
           1   125.6   131.2   114.2   175.8   135.3   124.6   153.9   203.9
           2   332.9   293.9   255.3   290.7   300.3   273.1   268.6   326.1
           3   309.7   375.8   350.2   342.1   302.8   310.6   367.4   324.5


Standard error: 28.41

========== Comparing means at the 5% level =========

Comparisons between Treatment means

         Mean
3       335.4  a
2       292.6  a
1       145.6  b

========= Summary of descriptive statistics ========

     Species           1                                 2
                Nobservd        Mean        s.d. Nobservd        Mean        s.d.
   Treatment
           1           3       125.6       13.90        3       131.2       24.11
           2           3       332.9       53.11        3       293.9       34.20
           3           3       309.7       28.56        3       375.8       71.54
      Margin           9       256.1      103.09        9       267.0      115.50

     Species           4                                 5
                Nobservd        Mean        s.d. Nobservd        Mean        s.d.
   Treatment
           1           3       114.2        8.56        3       175.8       61.68
           2           3       255.3       47.97        3       290.7        7.19
           3           3       350.2       34.21        3       342.1       68.37
      Margin           9       239.9      107.05        9       269.5       87.01

     Species           6                                 7
                Nobservd        Mean        s.d. Nobservd        Mean        s.d.
   Treatment
           1           3       135.3       22.72        3       124.6       11.41
           2           3       300.3       30.57        3       273.1       88.33
           3           3       302.8       79.17        3       310.6       21.23
      Margin           9       246.1       94.03        9       236.1       96.69

     Species           8                                 9
                Nobservd        Mean        s.d. Nobservd        Mean        s.d.
   Treatment
           1           3       153.9       32.71        3       203.9       37.56
           2           3       268.6       26.31        3       326.1       12.87
           3           3       367.4      114.90        3       324.5       52.08
```

```
      Margin         9      263.3     110.93            9      284.8      68.97

     Species    Margin
              Nobservd       Mean       s.d.
   Treatment
           1        24      145.6      39.06
           2        24      292.6      45.37
           3        24      335.4      60.78
      Margin        72      257.9      95.18



===== REML split-plot factorial analysis on WUE for season Spring =====

REML variance components analysis

Response variate:  WUE
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WUE

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                 Wald statistic   n.d.f.   F statistic   d.d.f.    F pr
Treatment                            0.21        2          0.10      6.0   0.903
Species                            126.26        7         18.04     42.0  <0.001
Treatment.Species                   22.29       14          1.59     42.0   0.122

Table of predicted means for Treatment

Treatment        1        2        3
             21.27    22.13    23.55
Standard error: 3.562

Table of predicted means for Species

Species        1        2        4        5        6        7        8        9
           22.38    30.98    13.65    45.63    29.78    17.76    11.49     6.87

Standard error: 3.469

Table of predicted means for Treatment.Species

     Species        1        2        4        5        6        7        8        9
   Treatment
           1    20.57    34.37    10.31    33.43    34.02    17.42    11.08     8.95
           2    17.09    30.71    20.64    53.98    21.98    22.73     6.74     3.19
```

```
         3   29.48   27.85    9.99   49.49   33.34   13.12   16.64    8.46

Standard error: 6.009

========== Comparing means at the 5% level =========

Comparisons between Species means

        Mean
5      45.63  a
2      30.98  b
6      29.78  bc
1      22.38  bcd
7      17.76  cde
4      13.65  de
8      11.49  de
9       6.87  e

========= Summary of descriptive statistics ========

   Species         1                         2
            Nobservd     Mean      s.d. Nobservd     Mean      s.d.
 Treatment
         1         3    20.57     8.179        3    34.37    12.920
         2         3    17.09     2.724        3    30.71     2.680
         3         3    29.48     4.355        3    27.85    10.487
    Margin         9    22.38     7.348        9    30.98     8.890

   Species         4                         5
            Nobservd     Mean      s.d. Nobservd     Mean      s.d.
 Treatment
         1         3    10.31     7.640        3    33.43    19.839
         2         3    20.64    18.439        3    53.98    13.429
         3         3     9.99     5.792        3    49.49    17.303
    Margin         9    13.65    11.641        9    45.63    17.489

   Species         6                         7
            Nobservd     Mean      s.d. Nobservd     Mean      s.d.
 Treatment
         1         3    34.02    11.135        3    17.42     2.529
         2         3    21.98     4.877        3    22.73    11.563
         3         3    33.34    22.420        3    13.12     1.603
    Margin         9    29.78    14.033        9    17.76     7.284

   Species         8                         9
            Nobservd     Mean      s.d. Nobservd     Mean      s.d.
 Treatment
         1         3    11.08     3.376        3     8.95     2.455
         2         3     6.74     4.957        3     3.19     1.810
         3         3    16.64     8.036        3     8.46     3.731
    Margin         9    11.49     6.604        9     6.87     3.669

   Species    Margin
            Nobservd     Mean      s.d.
 Treatment
```

```
      1     24    21.27   13.652
      2     24    22.13   16.909
      3     24    23.55   16.501
 Margin     72    22.32   15.559
```

A4 Statistical analysis of eight *Poaceae* species exposed to three water treatments from 1 Dec 2014 – 30 Nov 2015 (Annual production)

```
Harvest 3 Total weight all Nov2016.rtf

WUE of large biomass grasses: TOTAL: SUMMER + AUTUMN + SPRING HARVEST 3

========== REML split-plot factorial analysis on total fresh weight with all species
==========

REML variance components analysis

Response variate:  TotalFW

Fixed model:       Constant + Treatment + Species + Treatment.Species

Random model:      Rep + Rep.wplot + Rep.wplot.splot

Number of units:   71 (1 units excluded due to zero weights or missing values)


Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term              Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                        42.94        2         21.47      4.0   0.007

Species                         737.05        7        105.29     41.0 <0.001

Treatment.Species               113.85       14          8.13     41.0 <0.001

Table of predicted means for Treatment

Treatment       1       2       3

            51617   79550   87931

Standard errors

Treatment       1       2       3

             5504    5526    5504
```

```
Standard errors

Average:                5511.

Maximum:                5526.

Minimum:                5504.

Table of predicted means for Species

Species       1         2         4         5         6         7         8         9

        95936112956  16268  72445  81154  89575  80548  35381

Standard errors

Species       1         2         4         5         6         7         8         9

          5264    5264    5264    5264    5264    5431    5264    5264

Standard errors

Average:                5284.

Maximum:                5431.

Minimum:                5264.

Table of predicted means for Treatment.Species

    Species        1         2         4         5         6         7         8         9

  Treatment

             1  54890  85697    8002  43822  74428  54013  61435  30653

             2130259119498  20928  84902  67333104653  75034  33792

             3102658133674  19872  88610101701110058105174  41697

Standard errors

  Species        1         2         4         5         6         7         8         9

Treatment

           1    7452    7452    7452    7452    7452    7452    7452    7452

           2    7452    7452    7452    7452    7452    8463    7452    7452

           3    7452    7452    7452    7452    7452    7452    7452    7452

Standard errors

Average:                7494.
```

```
Maximum:            8463.

Minimum:            7452.

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

         Mean

3       87931   a

2       79550   a

1       51617   b

Comparisons between Species means

         Mean

2      112956   a

1       95936   b

7       89575   bc

6       81154   cd

8       80548   cd

5       72445   d

9       35381   e

4       16268   f

Comparisons between Treatment.Species means

           Mean

3 2      133674   a

2 1      130259   ab

2 2      119498   abc

3 7      110058   abcd

3 8      105174   abcde

2 7      104653   abcdef

3 1      102658   abcdefg

3 6      101701   abcdefg
```

```
3 5      88610  abcdefgh
1 2      85697  abcdefghi
2 5      84902  abcdefghij
2 8      75034  acdefghijk
1 6      74428  abcdefghijkl
2 6      67333  defghijklm
1 8      61435  cdefghijklmn
1 1      54890  defghijklmno
1 7      54013  defghijklmno
1 5      43822  efghijklmno
3 9      41697  fhijklmno
2 9      33792  hijklmno
1 9      30653  hjklmno
2 4      20928  lmno
3 4      19872  klmno
1 4       8002  mo
   ============= Summary of data for SDs ============
     Species          1                             2
               Nobservd      Mean        s.d. Nobservd      Mean        s.d.
   Treatment
           1          3     54890        9687        3     85697        8021
           2          3    130259       20297        3    119498       27002
           3          3    102658       12957        3    133674       17964
      Margin          9     95936       35482        9    112956       27105
     Species          4                             5
               Nobservd      Mean        s.d. Nobservd      Mean        s.d.
   Treatment
           1          3      8002        6180        3     43822        7236
```

```
         2         3     20928     12121         3     84902     17383

         3         3     19872     11119         3     88610      9696

    Margin         9     16268     10762         9     72445     23990

   Species         6                             7

            Nobservd      Mean      s.d.  Nobservd      Mean      s.d.

 Treatment

         1         3     74428     14515         3     54013      6618

         2         3     67333     10387         2    110211     14653

         3         3    101701      9599         3    110058      8468

    Margin         9     81154     18698         8     89080     30114

   Species         8                             9

            Nobservd      Mean      s.d.  Nobservd      Mean      s.d.

 Treatment

         1         3     61435      4865         3     30653      6624

         2         3     75034      9180         3     33792      1956

         3         3    105174      7389         3     41697     21587

    Margin         9     80548     20407         9     35381     12358

   Species    Margin

            Nobservd      Mean      s.d.

 Treatment

         1        24     51617     24435

         2        23     78942     40083

         3        24     87931     37852

    Margin        71     72744     37638

========== REML split-plot factorial analysis on total dry weight with all species
==========

REML variance components analysis

Response variate:  TotalDW

Fixed model:       Constant + Treatment + Species + Treatment.Species
```

```
Random model:     Rep + Rep.wplot + Rep.wplot.splot

Number of units:  71 (1 units excluded due to zero weights or missing values

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                 Wald statistic   n.d.f.   F statistic   d.d.f.    F pr

Treatment                           30.68        2         15.34      4.0   0.013

Species                            789.44        7        112.78     41.0 <0.001

Treatment.Species                  130.62       14          9.33     41.0 <0.001

Table of predicted means for Treatment

Treatment       1       2       3

            15639   21887   24305

Standard errors

Treatment       1       2       3

             1506    1512    1506

Standard errors

Average:               1508.

Maximum:               1512.

Minimum:               1506.

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

          28617   27584    5771   23857   28982   24897   16111    9064

Standard errors

Species       1       2       4       5       6       7       8       9

           1435    1435    1435    1435    1435    1482    1435    1435

Standard errors

Average:               1441.
```

```
Maximum:              1482.

Minimum:              1435.

Table of predicted means for Treatment.Species

    Species       1       2       4       5       6       7       8       9

  Treatment

          1   18019   22899    3240   15497   29313   15365   12255    8525

          2   37367   28617    6505   27279   21844   28521   16359    8603

          3   30466   31237    7569   28795   35788   30805   19720   10063

Standard errors

  Species       1       2       4       5       6       7       8       9

Treatment

        1    2049    2049    2049    2049    2049    2049    2049    2049

        2    2049    2049    2049    2049    2049    2330    2049    2049

        3    2049    2049    2049    2049    2049    2049    2049    2049

Standard errors

Average:              2061.

Maximum:              2330.

Minimum:              2049.

   ========== Comparing means at the 5% level =========

Comparisons between Treatment means

        Mean

3      24305   a

2      21887   a

1      15639   b

Comparisons between Species means

        Mean

6      28982   a

1      28617   ab
```

```
2        27584   abc

7        24897   bc

5        23857   c

8        16111   d

9         9064   e

4         5771   e

Comparisons between Treatment.Species means

              Mean

2 1        37367   a

3 6        35788   ab

3 2        31237   abc

3 7        30805   abc

3 1        30466   abc

1 6        29313   abc

3 5        28795   abcd

2 2        28617   abcd

2 7        28521   abcde

2 5        27279   abcdef

1 2        22899   abcdefg

2 6        21844   bcdefgh

3 8        19720   cdefghi

1 1        18019   cdefghij

2 8        16359   cdefghijk

1 5        15497   cdefghijk

1 7        15365   cdefghijk

1 8        12255   defghijk

3 9        10063   eghijk

2 9         8603   ghijk
```

```
1 9        8525  hijk

3 4        7569  ghijk

2 4        6505  gijk

1 4        3240  ik

   ============ Summary of data for SDs ===========

     Species        1                          2

             Nobservd      Mean       s.d. Nobservd      Mean       s.d.

   Treatment

           1        3     18019       2057        3     22899       2448

           2        3     37367       6524        3     28617       5225

           3        3     30466       3626        3     31237       3707

      Margin        9     28617       9333        9     27584       5039



     Species        4                          5

             Nobservd      Mean       s.d. Nobservd      Mean       s.d.

   Treatment

           1        3      3240       2659        3     15497       1945

           2        3      6505       4089        3     27279       5028

           3        3      7569       3914        3     28795       2252

      Margin        9      5771       3687        9     23857       6948

     Species        6                          7

             Nobservd      Mean       s.d. Nobservd      Mean       s.d.

   Treatment

           1        3     29313       5734        3     15365       1826

           2        3     21844       3687        2     30157       6063

           3        3     35788       3585        3     30805       1802

      Margin        9     28982       7166        8     24853       8303
```

```
     Species         8                         9
             Nobservd      Mean      s.d. Nobservd      Mean      s.d.
   Treatment
           1         3     12255      1300        3      8525      2005
           2         3     16359      2549        3      8603       609
           3         3     19720       852        3     10063      3577
      Margin         9     16111      3566        9      9064      2204
     Species    Margin
             Nobservd      Mean      s.d.
   Treatment
           1        24     15639      8102
           2        23     21741     11100
           3        24     24305     10439
      Margin        71     20545     10472
========== REML split-plot factorial analysis on total dry matter % with    all    species
==========
REML variance components analysis
Response variate:  TotalDM %
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep.wplot.splot
Number of units:   71 (1 units excluded due to zero weights or missing values)
Rep.wplot.splot used as residual term
Sparse algorithm with AI optimisation
Tests for fixed effects
Sequentially adding terms to fixed model
Fixed term                Wald statistic  n.d.f.   F statistic  d.d.f.    F pr
Treatment                          29.17       2         14.59    47.0 <0.001
Species                           367.45       7         52.49    47.0 <0.001
Treatment.Species                  36.72      14          2.62    47.0   0.007
```

```
Table of predicted means for Treatment

Treatment       1       2       3

            31.35   27.69   29.16

Standard errors

Treatment       1       2       3

             0.48    0.49    0.48

Standard errors

Average:            0.4808

Maximum:            0.4906

Minimum:            0.4760

Table of predicted means for Species

Species       1       2       4       5       6       7       8       9

          30.48   24.73   36.25   33.44   35.71   27.91   20.16   26.51


Standard errors

Species       1       2       4       5       6       7       8       9

           0.78    0.78    0.78    0.78    0.78    0.84    0.78    0.78

Standard errors

Average:            0.7850

Maximum:            0.8395

Minimum:            0.7772

Table of predicted means for Treatment.Species

    Species       1       2       4       5       6       7       8       9

  Treatment

          1   33.10   26.71   39.81   35.52   39.50   28.45   19.93   27.79

          2   28.64   24.09   29.71   32.22   32.40   27.24   21.76   25.46

          3   29.71   23.40   39.22   32.59   35.24   28.03   18.80   26.28

Standard errors
```

```
  Species       1      2      4      5      6      7      8      9
Treatment
        1    1.35   1.35   1.35   1.35   1.35   1.35   1.35   1.35
        2    1.35   1.35   1.35   1.35   1.35   1.65   1.35   1.35
        3    1.35   1.35   1.35   1.35   1.35   1.35   1.35   1.35
Standard errors
Average:             1.359
Maximum:             1.649
Minimum:             1.346
   ========== Comparing means at the 5% level =========
Comparisons between Treatment means
       Mean
1      31.35  a
3      29.16  b
2      27.69  c
Comparisons between Species means
       Mean
4      36.25  a
6      35.71  a
5      33.44  ab
1      30.48  bc
7      27.91  cd
9      26.51  d
2      24.73  d
8      20.16  e
Comparisons between Treatment.Species means
         Mean
1 4      39.81   a
```

```
1 6        39.50  ab

3 4        39.22  ab

1 5        35.52  abc

3 6        35.24  abcd

1 1        33.10  abcde

3 5        32.59  abcdef

2 6        32.40  bcdef

2 5        32.22  bcdef

3 1        29.71  cdefg

2 4        29.71  cdefg

2 1        28.64  cdefgh

1 7        28.45  cdefgh

3 7        28.03  defgh

1 9        27.79  efgh

2 7        27.24  defghi

1 2        26.71  efghi

3 9        26.28  efghi

2 9        25.46  fghij

2 2        24.09  ghij

3 2        23.40  ghij

2 8        21.76  hij

1 8        19.93  ij

3 8        18.80  j

   ============ Summary of data for SDs ============

    Species            1                          2

              Nobservd     Mean       s.d. Nobservd     Mean       s.d.

  Treatment

          1          3    33.10      2.778        3    26.71      0.769
```

```
         2          3      28.64      0.913          3      24.09      1.164
         3          3      29.71      0.974          3      23.40      0.374
    Margin          9      30.48      2.541          9      24.73      1.674
   Species          4                                5
             Nobservd       Mean       s.d.   Nobservd       Mean       s.d.
 Treatment
         1          3      39.81      2.509          3      35.52      1.844
         2          3      29.71      4.233          3      32.22      1.347
         3          3      39.22      3.074          3      32.59      1.758
    Margin          9      36.25      5.704          9      33.44      2.129
   Species          6                                7
             Nobservd       Mean       s.d.   Nobservd       Mean       s.d.
 Treatment
         1          3      39.50      4.110          3      28.45      0.275
         2          3      32.40      0.444          2      27.24      1.880
         3          3      35.24      2.663          3      28.03      1.218
    Margin          9      35.71      3.953          8      27.99      1.098
   Species          8                                9
             Nobservd       Mean       s.d.   Nobservd       Mean       s.d.
 Treatment
         1          3      19.93      1.208          3      27.79      1.587
         2          3      21.76      0.987          3      25.46      0.889
         3          3      18.80      1.380          3      26.28      6.249
    Margin          9      20.16      1.657          9      26.51      3.411
   Species     Margin
             Nobservd       Mean       s.d.
 Treatment
         1         24      31.35      6.826
```

```
          2        23      27.71      4.011

          3        24      29.16      6.683

     Margin        71      29.43      6.108

===== REML split-plot factorial analysis on WU for season Annual =====

REML variance components analysis

Response variate:  WU
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WU

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                  Wald statistic   n.d.f.    F statistic   d.d.f.     F pr
Treatment                           567.33        2         283.67      4.0   <0.001
Species                               2.31        7           0.33     42.0    0.936
Treatment.Species                    19.76       14           1.41     42.0    0.191

Table of predicted means for Treatment

Treatment       1        2        3
            634.4    842.0    998.2


Standard error: 11.25

Table of predicted means for Species

Species       1        2        4        5        6        7        8        9
          815.3    825.7    837.9    817.3    831.8    836.0    821.1    813.6

Standard error: 16.96

Table of predicted means for Treatment.Species

    Species        1        2        4        5        6        7        8        9
  Treatment
          1    623.9    690.2    635.7    620.2    598.5    665.5    615.6    625.4
          2    867.5    778.9    859.2    849.8    859.5    855.7    820.0    845.2
          3    954.5   1007.9   1018.7    982.0   1037.6    986.9   1027.8    970.4

Standard error: 29.07
```

```
========== Comparing means at the 5% level =========

Comparisons between Treatment means

         Mean
3       998.2  a
2       842.0  b
1       634.4  c


========= Summary of descriptive statistics ========

     Species         1                      2
            Nobservd    Mean    s.d. Nobservd    Mean    s.d.
   Treatment
           1         3   623.9   24.78        3   690.2   48.86
           2         3   867.5   63.40        3   778.9   48.44
           3         3   954.5  103.16        3  1007.9   48.60
      Margin         9   815.3  160.78        9   825.7  148.06

     Species         4                      5
            Nobservd    Mean    s.d. Nobservd    Mean    s.d.
   Treatment
           1         3   635.7   60.23        3   620.2   46.45
           2         3   859.2   39.14        3   849.8   13.11
           3         3  1018.7   46.17        3   982.0   35.20
      Margin         9   837.9  171.99        9   817.3  161.33

     Species         6                      7
            Nobservd    Mean    s.d. Nobservd    Mean    s.d.
   Treatment
           1         3   598.5   15.27        3   665.5   44.31
           2         3   859.5   55.74        3   855.7   59.52
           3         3  1037.6   22.00        3   986.9   84.83
      Margin         9   831.8  193.75        9   836.0  150.84

     Species         8                      9
            Nobservd    Mean    s.d. Nobservd    Mean    s.d.
   Treatment
           1         3   615.6   31.02        3   625.4   14.59
           2         3   820.0   10.48        3   845.2   72.20
           3         3  1027.8   40.75        3   970.4   53.91
      Margin         9   821.1  180.40        9   813.6  157.98

     Species    Margin
            Nobservd    Mean    s.d.
   Treatment
           1        24   634.4   43.09
           2        24   842.0   50.22
           3        24   998.2   57.21
      Margin        72   824.9  158.12


===== REML split-plot factorial analysis on WUE for season Annual =====
```

```
REML variance components analysis

Response variate:  WUE
Fixed model:       Constant + Treatment + Species + Treatment.Species
Random model:      Rep + Rep.wplot + Rep.wplot.splot
Number of units:   72

Rep.wplot.splot used as residual term

Sparse algorithm with AI optimisation
Analysis is subject to the restriction on WUE

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term                 Wald statistic   n.d.f.    F statistic   d.d.f.     F pr
Treatment                            0.79        2           0.39      4.0    0.698
Species                            321.44        7          45.92     42.0   <0.001
Treatment.Species                   41.84       14           2.99     42.0    0.003

Table of predicted means for Treatment

Treatment        1         2         3
             24.02     26.11     24.25

Standard error: 2.455

Table of predicted means for Species

Species         1         2         4         5         6         7         8         9
            35.09     33.18      6.65     26.98     39.05     26.03     19.67     11.71

Standard error: 2.506

Table of predicted means for Treatment.Species

       Species          1         2         4         5         6         7         8         9
     Treatment
             1      28.83     33.27      4.98     21.55     48.86     21.18     19.87     13.65
             2      43.11     36.86      7.43     30.00     32.18     29.09     19.96     10.27
             3      33.33     29.42      7.53     29.40     36.11     27.81     19.19     11.20

Standard error: 3.672

========== Comparing means at the 5% level =========

Comparisons between Species means

        Mean
6       39.05   a
1       35.09   a
2       33.18   ab
5       26.98   bc
7       26.03   bc
```

```
8       19.67  c
9       11.71  d
4        6.65  d

========= Summary of descriptive statistics ========

    Species         1                          2
             Nobservd     Mean     s.d. Nobservd     Mean      s.d.
  Treatment
          1         3    28.83    2.162        3    33.27     3.945
          2         3    43.11    7.211        3    36.86     7.030
          3         3    33.33    3.429        3    29.42     5.119
     Margin         9    35.09    7.558        9    33.18     5.759

    Species         4                          5
             Nobservd     Mean     s.d. Nobservd     Mean      s.d.
  Treatment
          1         3     4.98    3.720        3    21.55     8.229
          2         3     7.43    4.540        3    30.00     9.647
          3         3     7.53    4.114        3    29.40     3.337
     Margin         9     6.65    3.797        9    26.98     7.724

    Species         6                          7
             Nobservd     Mean     s.d. Nobservd     Mean      s.d.
  Treatment
          1         3    48.86    8.623        3    21.18     5.731
          2         3    32.18   14.760        3    29.09     9.195
          3         3    36.11    6.201        3    27.81    10.425
     Margin         9    39.05   11.817        9    26.03     8.369

    Species         8                          9
             Nobservd     Mean     s.d. Nobservd     Mean      s.d.
  Treatment
          1         3    19.87    1.191        3    13.65     3.283
          2         3    19.96    3.234        3    10.27     1.637
          3         3    19.19    0.357        3    11.20     2.358
     Margin         9    19.67    1.771        9    11.71     2.655

    Species    Margin
             Nobservd     Mean     s.d.
  Treatment
          1        24    24.02   13.390
          2        24    26.11   13.785
          3        24    24.25   10.897
     Margin        72    24.80   12.609
```

Table A1: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *P. maximum* (S1) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	LAI	DMY	DM %	CV	EY	WU	WUE	WEPE
			tons ha^{-1} yr^{-1}	m^{2} m^{-2}	tons ha^{-1} yr^{-1}	%	MJ kg^{-1}	GJ ha^{-1} yr^{-1}	mm	kg DM ha^{-1} mm^{-1}	MJ mm^{-1} ha^{-1} yr^{-1}
C1	T1	H1	13.19	0.68	2.08	15.86	N/A	N/A	N/A	N/A	N/A
C1	T1	H2	33.22	4.49	7.39	22.25	N/A	N/A	N/A	N/A	N/A
C1	T1	H3	38.01	2.79	13.29	35.24	16.45	218.59	389.07	34.05	561.83
C1	T2	H1	10.06	0.43	1.41	14.13	N/A	N/A	N/A	N/A	N/A
C1	T2	H2	36.47	6.46	7.55	20.69	N/A	N/A	N/A	N/A	N/A
C1	T2	H3	92.13	6.06	27.01	29.27	15.95	430.86	418.13	64.30	1030.44
C1	T3	H1	10.43	0.43	1.79	17.35	N/A	N/A	N/A	N/A	N/A
C1	T3	H2	33.18	3.80	6.45	19.43	N/A	N/A	N/A	N/A	N/A
C1	T3	H3	63.90	4.27	18.86	29.49	15.51	292.48	453.80	45.12	644.51
C2	T1	H1	8.13	0.74	1.74	21.36	N/A	N/A	N/A	N/A	N/A
C2	T1	H2	7.19	0.65	2.40	33.39	N/A	N/A	N/A	N/A	N/A
C2	T1	H3	6.80	0.69	2.13	31.41	16.45	35.00	96.02	22.19	364.49
C2	T2	H1	10.30	0.79	1.76	17.04	N/A	N/A	N/A	N/A	N/A
C2	T2	H2	11.60	1.37	3.50	30.22	N/A	N/A	N/A	N/A	N/A
C2	T2	H3	18.59	2.10	4.65	26.47	15.95	74.24	131.00	35.41	550.09
C2	T3	H1	5.95	0.56	1.06	17.87	N/A	N/A	N/A	N/A	N/A
C2	T3	H2	7.60	0.78	2.35	30.94	N/A	N/A	N/A	N/A	N/A
C2	T3	H3	8.69	1.16	2.55	29.41	15.51	39.52	206.91	12.40	190.98
C3	T1	H1	2.09	0.35	0.56	26.62	N/A	N/A	N/A	N/A	N/A
C3	T1	H2	2.16	0.15	0.72	33.41	N/A	N/A	N/A	N/A	N/A
C3	T1	H3	10.08	0.70	2.60	25.62	16.45	42.85	125.63	20.57	341.11
C3	T2	H1	7.83	0.75	1.73	22.11	N/A	N/A	N/A	N/A	N/A
C3	T2	H2	7.15	0.54	1.91	26.76	N/A	N/A	N/A	N/A	N/A
C3	T2	H3	19.53	1.34	5.71	29.04	15.95	91.02	332.90	17.09	273.43
C3	T3	H1	6.86	0.61	1.55	22.57	N/A	N/A	N/A	N/A	N/A
C3	T3	H2	11.73	1.00	3.23	27.54	N/A	N/A	N/A	N/A	N/A
C3	T3	H3	30.07	2.11	9.06	30.28	15.51	140.54	309.70	29.48	453.78

Table A2: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *P. purpureum* (S2) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^{2} m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**40.00**	**2.20**	**5.08**	**12.70**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**56.95**	**3.83**	**9.05**	**15.35**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**60.87**	**3.22**	**17.09**	**27.99**	**15.61**	**266.74**	**454.14**	**37.54**	**587.36**
C1	**T2**	**H1**	**36.78**	**2.36**	**5.49**	**14.99**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**66.11**	**3.91**	**10.43**	**15.77**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**63.15**	**2.76**	**17.00**	**27.04**	**15.11**	**256.82**	**364.90**	**47.67**	**703.81**
C1	**T3**	**H1**	**33.93**	**1.89**	**4.42**	**12.91**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**72.48**	**3.84**	**10.39**	**14.34**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**68.74**	**2.54**	**19.18**	**27.87**	**15.66**	**300.42**	**497.63**	**35.75**	**603.70**
C2	**T1**	**H1**	**1.13**	**0.12**	**0.15**	**13.45**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**2.63**	**0.25**	**0.79**	**30.08**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**6.40**	**0.77**	**1.49**	**23.36**	**15.61**	**23.20**	**98.18**	**14.96**	**236.29**
C2	**T2**	**H1**	**1.41**	**0.16**	**0.16**	**11.18**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**2.99**	**0.32**	**0.80**	**26.66**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**14.04**	**1.43**	**2.62**	**19.08**	**15.11**	**39.58**	**161.56**	**16.18**	**245.00**
C2	**T3**	**H1**	**1.79**	**0.18**	**0.22**	**12.18**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**2.46**	**0.24**	**0.63**	**25.82**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**11.10**	**1.05**	**1.95**	**17.60**	**15.66**	**30.48**	**202.04**	**9.64**	**150.85**
C3	**T1**	**H1**	**6.77**	**0.50**	**1.56**	**23.02**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**12.11**	**1.23**	**2.85**	**23.50**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**18.43**	**1.17**	**4.33**	**23.69**	**15.61**	**67.56**	**131.20**	**34.37**	**514.95**
C3	**T2**	**H1**	**9.40**	**0.74**	**1.86**	**19.82**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**22.79**	**1.64**	**4.00**	**17.53**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**42.31**	**2.46**	**9.00**	**21.47**	**15.11**	**135.89**	**293.91**	**30.71**	**462.37**
C3	**T3**	**H1**	**10.73**	**0.93**	**2.13**	**19.83**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**25.09**	**2.46**	**4.58**	**18.26**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**53.83**	**2.86**	**10.11**	**18.86**	**15.66**	**158.40**	**375.77**	**27.85**	**421.52**

Table A3: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *M. giganteus* (S4) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	LAI	DMY	DM %	CV	EY	WU	WUE	WEPE
			tons ha^{-1} yr^{-1}	m^{2} m^{-2}	tons ha^{-1} yr^{-1}	%	MJ kg^{-1}	GJ ha^{-1} yr^{-1}	mm	kg DM ha^{-1} mm^{-1}	MJ mm^{-1} ha^{-1} yr^{-1}
C1	**T1**	**H1**	**4.06**	**0.08**	**0.85**	**21.06**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**3.12**	**0.17**	**0.82**	**26.48**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**4.30**	**0.18**	**1.94**	**44.08**	**17.08**	**33.08**	**404.00**	**4.62**	**81.99**
C1	**T2**	**H1**	**4.48**	**0.08**	**0.84**	**18.75**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**5.33**	**0.22**	**1.36**	**25.58**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**3.97**	**0.07**	**1.56**	**39.50**	**16.73**	**26.08**	**393.72**	**3.78**	**66.25**
C1	**T3**	**H1**	**3.82**	**0.05**	**0.67**	**17.81**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**6.56**	**0.32**	**1.54**	**23.55**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**9.33**	**0.52**	**3.55**	**37.67**	**16.45**	**58.47**	**462.28**	**7.92**	**126.49**
C2	**T1**	**H1**	**0.28**	**0.01**	**0.06**	**22.74**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**0.25**	**0.01**	**0.12**	**49.60**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**0.23**	**0.02**	**0.08**	**37.07**	**17.08**	**1.43**	**101.66**	**0.84**	**14.03**
C2	**T2**	**H1**	**0.62**	**0.04**	**0.15**	**24.10**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**0.56**	**0.03**	**0.26**	**47.63**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**0.60**	**0.05**	**0.17**	**29.89**	**16.73**	**2.89**	**179.05**	**0.97**	**16.12**
C2	**T3**	**H1**	**0.49**	**0.04**	**0.10**	**21.27**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**0.57**	**0.03**	**0.28**	**48.53**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**1.71**	**0.18**	**0.51**	**31.45**	**16.45**	**8.46**	**229.33**	**2.28**	**36.87**
C3	**T1**	**H1**	**2.98**	**0.10**	**0.67**	**22.61**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**3.78**	**0.23**	**1.15**	**30.43**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**3.48**	**0.13**	**1.22**	**34.34**	**17.08**	**20.84**	**114.20**	**10.31**	**182.43**
C3	**T2**	**H1**	**4.58**	**0.20**	**0.92**	**20.08**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**7.93**	**0.41**	**2.47**	**31.22**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**16.36**	**0.35**	**4.77**	**27.12**	**16.73**	**79.88**	**255.28**	**20.64**	**312.90**
C3	**T3**	**H1**	**4.63**	**0.18**	**1.14**	**24.54**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**8.10**	**0.38**	**2.38**	**29.33**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**8.84**	**0.24**	**3.50**	**39.90**	**16.45**	**57.57**	**350.19**	**9.99**	**164.39**

Table A4: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *C. zizanoides* (S5) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^{2} m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**8.30**	**0.36**	**1.92**	**23.39**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**9.72**	**0.62**	**2.43**	**25.02**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**21.55**	**0.85**	**7.95**	**36.92**	**16.75**	**133.17**	**389.88**	**16.09**	**341.56**
C1	**T2**	**H1**	**7.89**	**0.33**	**1.78**	**22.49**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**9.56**	**0.54**	**2.27**	**23.71**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**29.57**	**1.29**	**8.93**	**30.35**	**16.25**	**145.12**	**405.18**	**18.00**	**358.15**
C1	**T3**	**H1**	**8.25**	**0.42**	**2.01**	**24.90**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**9.19**	**0.55**	**2.17**	**23.64**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**28.36**	**1.27**	**9.45**	**32.82**	**16.21**	**153.13**	**442.22**	**21.43**	**346.28**
C2	**T1**	**H1**	**2.69**	**0.08**	**0.61**	**22.56**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**2.16**	**0.08**	**0.88**	**40.80**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**5.56**	**0.22**	**1.88**	**33.78**	**16.75**	**31.45**	**98.28**	**19.21**	**319.99**
C2	**T2**	**H1**	**3.42**	**0.15**	**0.71**	**20.64**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**3.11**	**0.12**	**1.18**	**37.99**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**8.45**	**0.38**	**2.68**	**31.70**	**16.25**	**43.51**	**179.69**	**15.11**	**242.14**
C2	**T3**	**H1**	**3.63**	**0.13**	**0.74**	**20.29**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**2.74**	**0.10**	**1.02**	**37.28**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**10.11**	**0.52**	**3.18**	**31.53**	**16.21**	**51.62**	**222.52**	**14.56**	**231.96**
C3	**T1**	**H1**	**7.95**	**0.76**	**2.26**	**28.37**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**11.84**	**0.74**	**3.76**	**31.77**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**16.72**	**0.39**	**5.67**	**34.26**	**16.75**	**94.98**	**175.78**	**33.43**	**540.36**
C3	**T2**	**H1**	**18.46**	**1.71**	**4.27**	**23.14**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**34.18**	**2.32**	**9.68**	**28.33**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**46.88**	**2.02**	**15.67**	**33.59**	**16.25**	**254.71**	**290.70**	**53.98**	**1009.13**
C3	**T3**	**H1**	**15.76**	**1.64**	**3.44**	**21.81**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**37.48**	**2.25**	**10.69**	**28.52**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**50.13**	**1.57**	**16.16**	**32.65**	**16.21**	**261.95**	**342.07**	**49.49**	**765.79**

Table A5: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *H. tamba* (S6) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^{2} m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**19.64**	**0.49**	**3.72**	**18.96**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**14.50**	**0.65**	**3.71**	**25.59**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**51.26**	**1.90**	**22.94**	**44.76**	**16.94**	**388.70**	**371.57**	**62.27**	**1046.09**
C1	**T2**	**H1**	**17.01**	**0.45**	**3.52**	**20.68**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**17.59**	**0.75**	**4.50**	**25.55**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**30.07**	**1.15**	**12.87**	**42.79**	**16.82**	**216.47**	**388.31**	**47.45**	**557.47**
C1	**T3**	**H1**	**26.53**	**0.76**	**5.18**	**19.53**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**20.26**	**1.02**	**5.14**	**25.36**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**57.72**	**2.61**	**25.38**	**43.97**	**16.22**	**411.53**	**502.91**	**50.10**	**818.29**
C2	**T1**	**H1**	**2.23**	**0.16**	**0.43**	**19.18**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**1.80**	**0.05**	**0.85**	**47.06**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**5.25**	**0.41**	**1.67**	**31.75**	**16.94**	**28.26**	**104.87**	**15.77**	**269.47**
C2	**T2**	**H1**	**1.52**	**0.08**	**0.27**	**17.72**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**2.97**	**0.14**	**1.17**	**39.33**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**8.55**	**0.76**	**2.32**	**27.11**	**16.82**	**39.00**	**179.39**	**13.02**	**217.41**
C2	**T3**	**H1**	**1.19**	**0.09**	**0.27**	**22.43**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**4.15**	**0.13**	**1.89**	**45.48**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**11.12**	**1.10**	**3.16**	**28.43**	**16.22**	**51.27**	**217.43**	**14.55**	**235.79**
C3	**T1**	**H1**	**4.88**	**0.28**	**1.51**	**31.00**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**12.93**	**0.91**	**3.74**	**28.93**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**17.92**	**0.31**	**4.71**	**26.31**	**16.94**	**79.86**	**135.30**	**34.02**	**692.56**
C3	**T2**	**H1**	**12.60**	**0.62**	**2.93**	**23.26**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**19.11**	**1.43**	**5.03**	**26.31**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**28.71**	**0.33**	**6.66**	**23.19**	**16.82**	**112.01**	**300.30**	**21.98**	**372.98**
C3	**T3**	**H1**	**11.88**	**0.73**	**3.03**	**25.50**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**24.21**	**1.22**	**7.39**	**30.53**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**32.86**	**0.28**	**7.28**	**22.17**	**16.22**	**118.12**	**302.83**	**33.34**	**390.07**

Table A6: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *B. brizantha* (S7) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^{2} m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**4.28**	**0.17**	**0.48**	**11.14**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**23.64**	**2.06**	**4.02**	**17.01**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**30.53**	**2.92**	**9.53**	**31.24**	**15.41**	**146.84**	**437.28**	**18.90**	**335.80**
C1	**T2**	**H1**	**5.74**	**0.27**	**0.60**	**10.64**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**19.78**	**1.93**	**3.45**	**17.43**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**63.20**	**5.12**	**18.93**	**29.74**	**16.05**	**303.74**	**415.36**	**36.13**	**731.28**
C1	**T3**	**H1**	**4.17**	**0.18**	**0.49**	**11.84**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**24.34**	**2.28**	**4.25**	**17.48**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**68.73**	**3.15**	**21.31**	**31.10**	**15.77**	**336.03**	**471.63**	**39.43**	**712.48**
C2	**T1**	**H1**	**10.22**	**1.22**	**1.88**	**18.43**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**19.09**	**1.91**	**5.84**	**30.58**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**14.33**	**2.48**	**3.68**	**25.59**	**15.41**	**56.68**	**96.20**	**38.66**	**589.16**
C2	**T2**	**H1**	**11.29**	**1.25**	**1.85**	**16.44**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**17.68**	**1.69**	**5.32**	**30.07**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**18.91**	**2.53**	**4.30**	**22.91**	**16.05**	**69.06**	**138.70**	**28.67**	**497.93**
C2	**T3**	**H1**	**7.44**	**0.73**	**1.50**	**20.18**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**13.55**	**1.36**	**4.17**	**30.76**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**23.15**	**3.66**	**5.40**	**23.36**	**15.77**	**85.08**	**215.19**	**21.71**	**395.38**
C3	**T1**	**H1**	**4.43**	**0.61**	**1.15**	**25.86**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**3.93**	**0.53**	**1.17**	**29.77**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**9.15**	**1.09**	**2.15**	**23.53**	**15.41**	**33.18**	**124.60**	**17.42**	**266.19**
C3	**T2**	**H1**	**6.20**	**0.86**	**1.39**	**22.46**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**2.59**	**0.18**	**0.59**	**22.87**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**28.10**	**2.97**	**6.93**	**24.45**	**16.05**	**111.19**	**273.10**	**22.73**	**407.12**
C3	**T3**	**H1**	**4.31**	**0.69**	**1.07**	**24.74**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**10.07**	**1.51**	**2.54**	**25.27**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**18.18**	**2.61**	**4.10**	**22.43**	**15.77**	**64.59**	**310.60**	**13.12**	**207.98**

Table A7: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *S. bicolor* (S8) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^2 m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**19.86**	**0.93**	**2.01**	**10.21**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**43.00**	**2.13**	**6.90**	**16.04**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**48.57**	**2.19**	**10.31**	**21.25**	**16.50**	**170.08**	**381.70**	**26.91**	**445.60**
C1	**T2**	**H1**	**19.62**	**0.99**	**2.29**	**11.67**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**50.23**	**2.63**	**8.46**	**16.84**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**58.70**	**2.53**	**13.86**	**23.50**	**15.84**	**219.59**	**406.73**	**34.10**	**539.91**
C1	**T3**	**H1**	**14.70**	**0.83**	**1.42**	**9.76**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**50.38**	**2.72**	**8.90**	**17.68**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**63.17**	**2.83**	**13.28**	**20.96**	**16.06**	**213.26**	**547.04**	**24.23**	**389.85**
C2	**T1**	**H1**	**0.34**	**0.04**	**0.06**	**17.05**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**0.95**	**0.12**	**0.26**	**27.14**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**1.18**	**0.11**	**0.27**	**23.02**	**16.50**	**4.51**	**92.00**	**2.93**	**46.23**
C2	**T2**	**H1**	**1.26**	**0.14**	**0.20**	**15.95**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**2.60**	**0.32**	**0.55**	**21.27**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**3.46**	**0.33**	**0.67**	**19.20**	**15.84**	**10.65**	**144.00**	**4.58**	**72.03**
C2	**T3**	**H1**	**1.52**	**0.20**	**0.25**	**16.21**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**2.19**	**0.27**	**0.48**	**21.86**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**4.24**	**0.32**	**0.83**	**19.21**	**16.06**	**13.25**	**183.00**	**4.60**	**71.71**
C3	**T1**	**H1**	**0.06**	**0.01**	**0.01**	**19.97**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**6.26**	**0.67**	**1.24**	**19.86**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**11.69**	**1.99**	**1.67**	**14.85**	**16.50**	**27.61**	**153.90**	**11.08**	**179.39**
C3	**T2**	**H1**	**0.04**	**0.00**	**0.01**	**23.81**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**3.60**	**0.40**	**0.64**	**17.70**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**12.88**	**2.07**	**1.82**	**12.92**	**15.84**	**28.88**	**268.59**	**6.74**	**107.51**
C3	**T3**	**H1**	**0.04**	**0.00**	**0.01**	**22.64**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**7.85**	**0.85**	**1.13**	**14.39**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**37.77**	**5.57**	**5.62**	**14.75**	**16.06**	**90.22**	**367.43**	**16.64**	**245.54**

Table A8: Fresh matter yield (FMY), leaf area index (LAI), dry matter yield (DMY), dry matter percentage (DM %), calorific value (CV), annual energy yield (EY), water use (WU), water use efficiency (WUE) and water to energy production efficiency (WEPE) of *S. bicolor* (S9) over three production cycles (C) from 1 Dec 2014 – 30 Nov 2015.

			FMY	**LAI**	**DMY**	**DM %**	**CV**	**EY**	**WU**	**WUE**	**WEPE**
			tons ha^{-1} yr^{-1}	**m^{2} m^{-2}**	**tons ha^{-1} yr^{-1}**	**%**	**MJ kg^{-1}**	**GJ ha^{-1} yr^{-1}**	**mm**	**kg DM ha^{-1} mm^{-1}**	**MJ mm^{-1} ha^{-1} yr^{-1}**
C1	**T1**	**H1**	**13.45**	**0.93**	**1.85**	**13.73**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H2**	**25.05**	**1.29**	**5.99**	**23.70**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T1**	**H3**	**20.12**	**1.49**	**6.42**	**31.80**	**16.29**	**104.52**	**372.84**	**17.28**	**280.33**
C1	**T2**	**H1**	**13.30**	**1.16**	**2.16**	**16.15**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H2**	**27.11**	**1.56**	**6.91**	**25.48**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T2**	**H3**	**20.13**	**1.52**	**5.95**	**29.57**	**16.26**	**96.71**	**408.08**	**14.80**	**236.98**
C1	**T3**	**H1**	**14.75**	**1.13**	**2.33**	**15.85**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H2**	**26.09**	**1.04**	**6.37**	**24.40**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C1	**T3**	**H3**	**19.84**	**1.51**	**6.19**	**31.28**	**16.19**	**100.31**	**414.42**	**14.94**	**242.06**
C2	**T1**	**H1**	**0.57**	**0.07**	**0.09**	**15.96**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H2**	**0.95**	**0.11**	**0.28**	**29.05**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T1**	**H3**	**1.25**	**0.10**	**0.27**	**23.09**	**16.29**	**4.36**	**108.00**	**2.65**	**39.68**
C2	**T2**	**H1**	**0.52**	**0.08**	**0.07**	**13.64**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H2**	**3.69**	**0.53**	**0.85**	**23.19**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T2**	**H3**	**7.16**	**0.57**	**1.61**	**22.39**	**16.26**	**26.24**	**187.00**	**8.72**	**138.56**
C2	**T3**	**H1**	**0.90**	**0.16**	**0.12**	**13.42**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H2**	**6.23**	**0.89**	**1.38**	**22.13**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C2	**T3**	**H3**	**10.30**	**0.89**	**2.37**	**22.81**	**16.19**	**38.35**	**225.00**	**8.34**	**168.31**
C3	**T1**	**H1**	**0.07**	**0.01**	**0.01**	**17.33**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H2**	**5.94**	**0.63**	**1.20**	**20.28**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T1**	**H3**	**9.29**	**1.56**	**1.84**	**19.52**	**16.29**	**29.95**	**203.90**	**8.95**	**146.90**
C3	**T2**	**H1**	**0.06**	**0.01**	**0.01**	**22.73**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H2**	**7.66**	**1.06**	**1.28**	**16.70**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T2**	**H3**	**6.50**	**1.14**	**1.04**	**15.82**	**16.26**	**16.96**	**326.10**	**3.19**	**52.02**
C3	**T3**	**H1**	**0.05**	**0.01**	**0.01**	**24.19**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H2**	**7.17**	**0.81**	**1.17**	**16.36**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
C3	**T3**	**H3**	**22.48**	**3.36**	**3.43**	**15.76**	**16.19**	**55.63**	**324.50**	**8.46**	**171.43**