Evaluation of Wastewaters to Provide Optimum Water and Nutrient Products for Growing Turf and Native Plants

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Abstract

The recent drought in South Eastern Australia and associated water restrictions led to a significant increase in the use of greywater for the watering of lawn and garden areas. There was also an emerging trend for sustainable drought resistant landscaping for which some Australian native plants were suitable. There however was an uncertainty about whether greywater was a useful source of water for plants or whether it could be harmful.

This study investigated how two varieties of turf Kikuyu *(Pennisetum Clandestinum)* and Tall Fescue *(Festuca arundinacea)*, and two varieties of Australian native flowers Scaly Buttons *(Leptorhynchos squamatus)* and Small Vanilla Lilies *(Arthropodium minus)* grew when watered with several different types of greywaters sourced from a bathroom shower and a laundry. The greywaters included shower water with and without urine, and total wash and deep rinse laundry waters made from phosphate containing and phosphate free detergents. The growths were compared against samples treated with water, and with a plant food (N:P:K ratio 15.0:13.1:12.4).

The turfs and native flowers were grown in pots and the growth of the turf was determined by weighing the dried clippings, and by measuring the growth heights between each cutting session. The growths of the native plants were determined by measuring the lengths of stems or leaves during the growth period, and after harvest by counting the number of flowers, by weighing the dried foliage and roots, and by measuring the lengths of the stems.

The conclusions were that the greywaters used were not harmful to the turf and the native plants. The addition of urine to greywaters significantly increased the growth of the turf and the native plants. Greywaters without added urine produced equal or better growth than water, however in the longer term the growth was limited by a lack of nutrients. Both varieties of native flowers produced very good growth with the plant food and were not harmed by the high phosphorus content.

Student Declaration

"I, Wieslaw Jan Zielinski, declare that the PhD thesis entitled 'Evaluation of Wastewaters to Provide Optimum Water and Nutrient Products for Growing Turf and Native Plants' is no more than 100,000 words in length including quotes and exclusive of tables, figures, charts, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature Date

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1.1 Introduction

The thesis presented here reports the findings of a practical research study that was carried out during the latter stage of the Millennium drought, which in south-eastern Australia occurred between the years 1997 to 2009. The research was conducted between 2007 and 2009 and involved the use of several types of greywaters to grow two types of turf grass, and two types of Australian native flowers. The plants were grown in pots that were placed out in the open, and so were subject to the weather conditions and to watering from any rain events, as well as watering from the greywater treatments. The greywaters were naturally produced by washing activities in the laundry and the shower. In addition, to study the effects of blends of water types, some blending was done to produce a mixture of laundry and shower waters, and to add urine to shower water. The urine was added to some greywaters at levels that were estimated to be likely if a person urinated while having a shower. These levels of dilution of urine were considerably greater than has normally been used for crop growth in various countries. The maximum addition of urine to shower water was 1% v/v.

The grass turf species used in this study were donated by H G Turf Pty Ltd as rolls of instant turf, and the two species were:

- Tall Fescue (*Festuca arundinacea*).
- Kikuyu (*Pennisetum clandestinum*).

The two native flower species investigated were purchased from the Iramoo Native Plant Nursery at Victoria University St Albans campus, and grown from local indigenous sources. The two species were:

- Scaly Buttons (*Leptorhynchos squamatus*).
- Small Vanilla Lilies (*Arthropodium minus*).

The greywater streams used in this research were:

- Shower water.

- Shower water plus urine.
- Total wash laundry water containing phosphate.
- Deep rinse laundry water containing phosphate.
- Total wash laundry water with no phosphate.
- Deep rinse laundry water with no phosphate.
- Blend of total wash phosphate laundry water and shower water.
- Blend of total wash phosphate laundry water and shower water plus urine.

The reference treatments for comparison against the greywater treatments were:

- Miracle-Gro® All Purpose plant food solution.
- Tap water

It should be noted that in this thesis the native plants used in the experiments are referred to as native flowers rather than native forbs (the more technically accurate term), because it is believed that 'flowers' is a more commonly understood term than 'forbs'.

Municipal wastewater normally produced by households and by community centres such as sports clubs is generally classified as blackwater or greywater, the type depending from which outlets the wastewater is sourced:

- **Blackwater** – wastewater grossly contaminated with human excrement discharges, which includes waters sourced from toilets, urinals, and bidets.
- **Greywater** – wastewater excluding toilet waste and may include water from showers, baths, hand basins, laundries, kitchen sinks and dishwashers.

This research was initially conceived during the Millennium drought, because it was obvious from just walking around the neighbourhood, that some householders were changing to drought resistant landscaping, and/or installing rainwater tanks, and/or using greywater. In the face of these ongoing dry conditions, many domestic households had begun collecting or diverting laundry and bathroom water directly onto gardens. The use of greywater appeared to be on a greater scale than was personally observed during the shorter droughts of 1972/73 and 1982/83. It was subsequently published by the Australian Bureau of Statistics (2010) that in 2007, 54% of Australian and 72% of Victorian households reported that they had used greywater. Greywater was also reported as being the most common main source of water for the garden by 24% of Australian and by 43% of Victorian households.

There was concern that greywater may contain ingredients that could be detrimental to plant growth, as reported by Jeppesen (1996). Early in the formulation of this research project, when the Millennium drought was into its sixth year, there was ongoing discussion in the broadcasting media as to the suitability of adding greywater to gardens, and whether greywater streams contained products that were likely to be harmful to plants or gardens. Some callers to talk back radio gardening programs were uncertain whether they could use untreated greywater on gardens, or on native plant species, and even on lawns. The callers were likely to be told to use greywater from the bathroom and only the rinse water from the laundry, and to purchase low salt and phosphate free laundry detergents. The recommendation to use the rinse water can also be found in publications such as *Sustainable Gardening in Brimbank* (Sustainable Gardening Australia et al 2006), or in *Grey water – recycling water at home* (Better Health Channel 2011). Using only the rinse water from the laundry would reduce the amounts of salts (including phosphates), surfactants, and other ingredients deposited to the soil, however it would basically make use of about half of the already limited volume of greywater produced in the laundry. Was it necessary to discard the laundry wash water and to only use the deep rinse water? Not apparently to 38% of respondents to a survey conducted in the Western Sydney Region, who said they used both waters (Pinto & Maheshwari 2008). The same survey also found that 38% of participants considered health risk to people, plants, and soil to be very important for any decision on reuse of greywater. Health risk and cost were however not very important for 10% of the survey participants.

Early literature search revealed that there was no obvious research, under Australian conditions, and little literature from overseas, that examined the impacts of unmixed greywater from the shower, or from the laundry, on the growth of plants in domestic gardens. Some information was available about using treated waste waters for growing golf course turf, using greywater for growing plants and crops, and about using urine as a plant fertiliser. In most cases greywater was considered to be a mixture of greywaters from several sources around the house. There was also a lack of information about whether greywater from specific sources in households or community centres could be used to grow certain turf and native flower species, especially during droughts. Mostly studies of the impacts of wastewater on gardens and lawns/turf assumed that the water had to be treated before it was used, and this involved mixing the greywater streams, treating so that nutrients were removed or reduced, and then dispersal onto gardens. Several studies of using either laundry or bathroom greywaters to grow plants in pots have been published since the field work for this study had been done. These studies differ from the work undertaken here, and are summarised in the literature review in Chapter 2.

In parallel with the emerging trend of using domestic greywater on amenity gardens, the more than decade long drought conditions between 1997 and 2009, and its associated water restrictions, resulted in an emerging trend for sustainable drought resistant landscaping, including use of some Australian native plants (Department of Environment and Natural Resources 2010; Sustainable Gardening Australia 2011). Australian native plants are regarded as being good water wise plants because once established, they require little water, very little maintenance, and need only low nutrient levels (Hahn 2008; Sustainable Gardening Australia et al 2006). No research literature was found on the effects of domestic greywater on native garden plants. It was also clear that many community facilities in Melbourne and elsewhere that generated large volumes of greywater (e.g. sports clubs, social venues) had surrounding garden amenity areas that were suffering, not just because of the drought but also because of the water restrictions that were in force. This in turn was affecting the suitability of the facilities as community venues, especially for outdoor functions over the summer period. The potential for using greywater from specific sources in households or community centres was in need of specific investigation.

Preliminary informal discussions in the local community revealed another issue surrounding greywater use that was not being discussed in these community conversations – the presence of urine in household wastewater streams. Bathroom sourced greywater may contain urine according to a report by NSW Health (2000) which states

> "…people often urinate in showers and baths…".

The writer can accept that some people may urinate while having a shower, but it is difficult to envisage that people may urinate into bath water. The bath however is not a major source of domestic greywater consuming around 2 litres per person per day (2 l/p/d) of water, compared to the 31 l/p/d of water consumed by the shower (Arthuraliya, Roberts & Brown 2012), so the entry of urine into greywater via the bath is likely to be relatively small. Urine in shower water can be viewed as either a contaminant, or if the shower water is being used for maintaining gardens or green spaces, as a potential source of plant nutrients (WHO 2006b). The writer does not recall ever hearing discussions on the radio gardening programs, that urine may sometimes be present in shower water or in mixed greywater.

1.2 Significance of the study

At the time of conducting this research there was no scientific study reported in the literature, in Australia or elsewhere, of the effects of untreated domestic greywater on the growth of amenity garden plants. The study reported here is a first level response to this gap in the research. The project was framed to test the feasibility of using greywater on domestic amenity plants, as a non-detrimental source of water, and also for its potential benefits of providing growth nutrients. To keep the study within manageable limits, a single source of ‘typical’ domestic greywater was used. Four ‘typical’ plant species were investigated, two domestic lawn grass species (one warm season grass and one cool season grass), and two local native plant species (one dicot species and one monocot species). The study makes no claim to be a comprehensive study of the range of domestic greywater combinations on domestic amenity plants – this will need to await further investigation. The study reported here has focused on the prior need to establish whether domestic greywater is a potential source of water and nutrients for domestic/community amenity gardens, and whether or not there are any detrimental or advantageous effects of using the various types of greywater (shower, washing machine, and blends of these) on the species investigated.

As a follow on from this study, there is the need for studies that look at the range of domestic greywater types on plant growths, and on a wider range of plants. The aims of the study outlined in the next section specifically address the key gaps in the literature at the time of the study:

- Is it feasible for ‘typical’ domestic greywater to be used as a source of water for amenity garden plant growth, and are there any detrimental effects on the plants?
- If domestic greywater is not detrimental to plant growth, does it provide some source of nutrients that contribute to plant growth?

In practical terms, the outcomes of this and follow up studies will be valuable in planning ways in which people can use domestic greywater on amenity gardens in Southern Australia during any future extended drought, as predicted by climate change modelling (CSIRO and Bureau of Meteorology 2015). Similar needs are predicted by climate change studies for many other parts of the world by international and country-specific climate change studies (IPCC 2014).

1.3 Aims of the research project

The major aims of the research project reported in this thesis were to determine:

1. The growth responses of the plants subjected to the different greywaters, and how the growth compared against samples receiving tap water, and those receiving tap water plus a commercially available all purpose water soluble plant food.
2. Whether greywater containing urine at levels that could result if people urinated while having a shower, would be suitable for frequent use in supplying the water and nutrient needs of recreational lawns and native flowers, especially during drought periods.

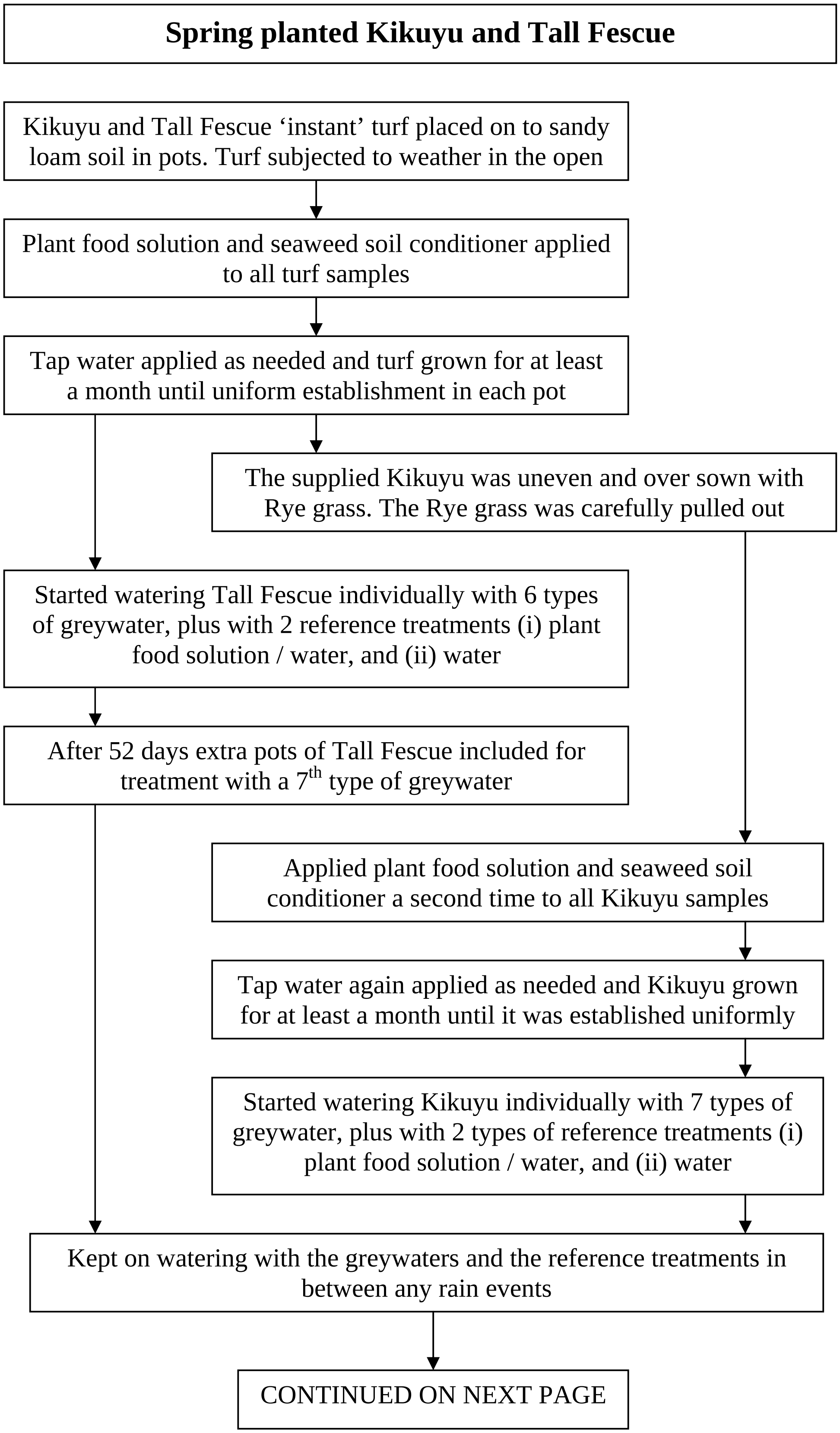
The work was also intended to provide answers to the following questions:

- The effect of the urine added to greywater on the growth of the turf and native plant species compared with water-alone and greywaters without added urine.
- Comparisons of growth of turf and native flowers resulting from using greywaters containing urine, and samples treated with the all purpose plant food.
- Differences in growth resulting from greywaters based on a phosphate free laundry detergent, and on a detergent containing phosphate.

- Differences in growth resulting from using total wash laundry greywaters, and using only the deep rinse greywaters.
- Whether the continued use of a commercial plant food containing 13.1% phosphorus and (N:P:K ratio 15.0:13.1:12.4), could be harmful to the native flower species selected for study.
- Whether the continued use of total wash or deep rinse greywaters based on a phosphate containing laundry detergent, could be harmful to the growth of native flower species.

1.4 Summaries of the procedures used to grow the plants

The summaries of how the turf and native flower field work experiments were carried out are presented in the three following flow charts (Figures 1.1 to 1.3). Detailed methodology for turf experiments can be found in Chapter 4, and for native flowers in Chapter 8.


Spring planted Kikuyu and Tall Fescue
Kikuyu and Tall Fescue 'instant' turf placed on to sandy loam soil in pots. Turf subjected to weather in the open
Plant food solution and seaweed soil conditioner applied to all turf samples
Tap water applied as needed and turf grown for at least a month until uniform establishment in each pot
The supplied Kikuyu was uneven and over sown with Rye grass. The Rye grass was carefully pulled out
Started watering Tall Fescue individually with 6 types of greywater, plus with 2 reference treatments (i) plant food solution / water, and (ii) water
After 52 days extra pots of Tall Fescue included for treatment with a 7th type of greywater
Applied plant food solution and seaweed soil conditioner a second time to all Kikuyu samples
Tap water again applied as needed and Kikuyu grown for at least a month until it was established uniformly
Started watering Kikuyu individually with 7 types of greywater, plus with 2 types of reference treatments (i) plant food solution / water, and (ii) water
Kept on watering with the greywaters and the reference treatments in between any rain events
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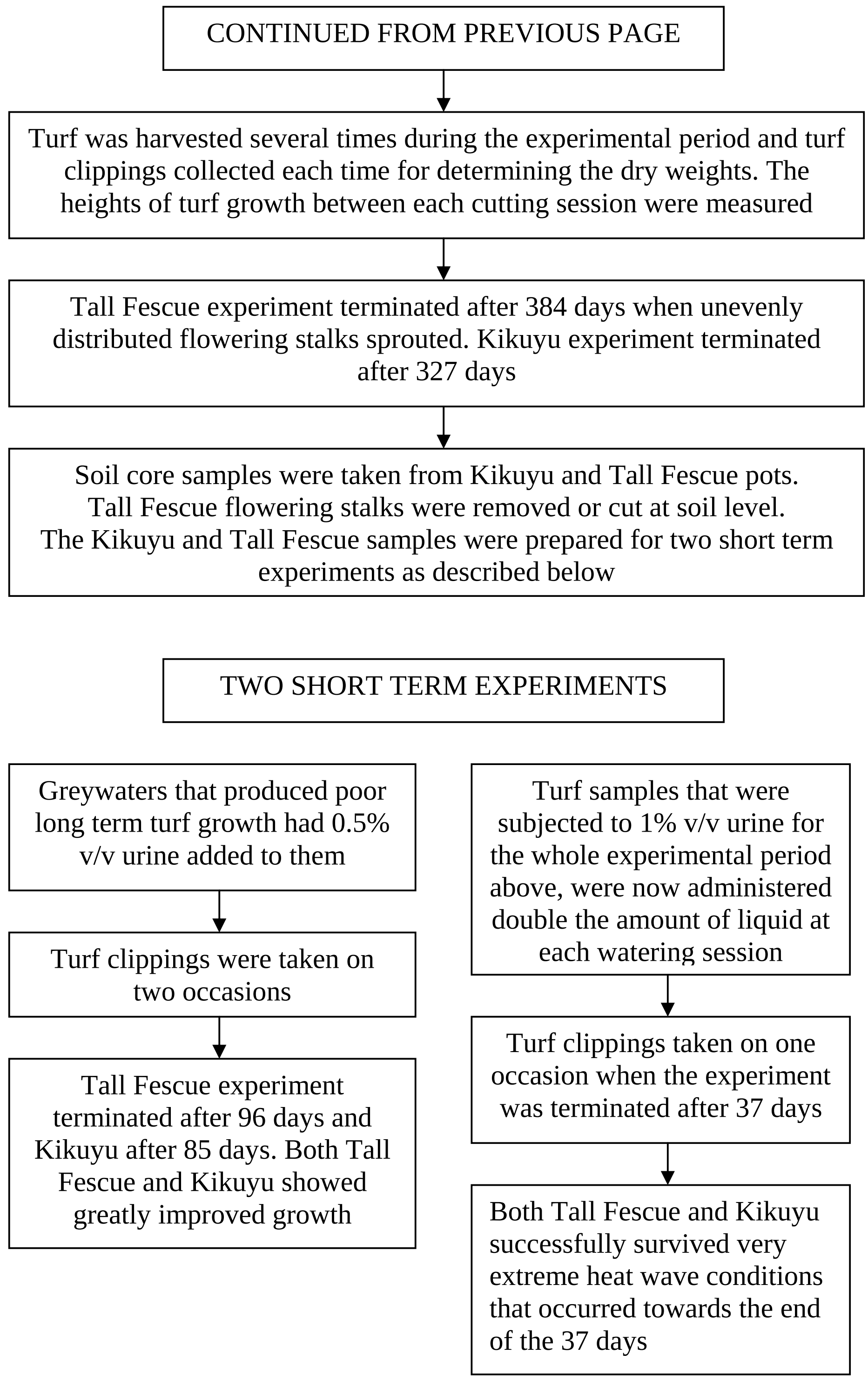

Figure 1.1 – Flow chart showing how the field work study with Spring planted Kikuyu and Tall Fescue turf grasses was carried out

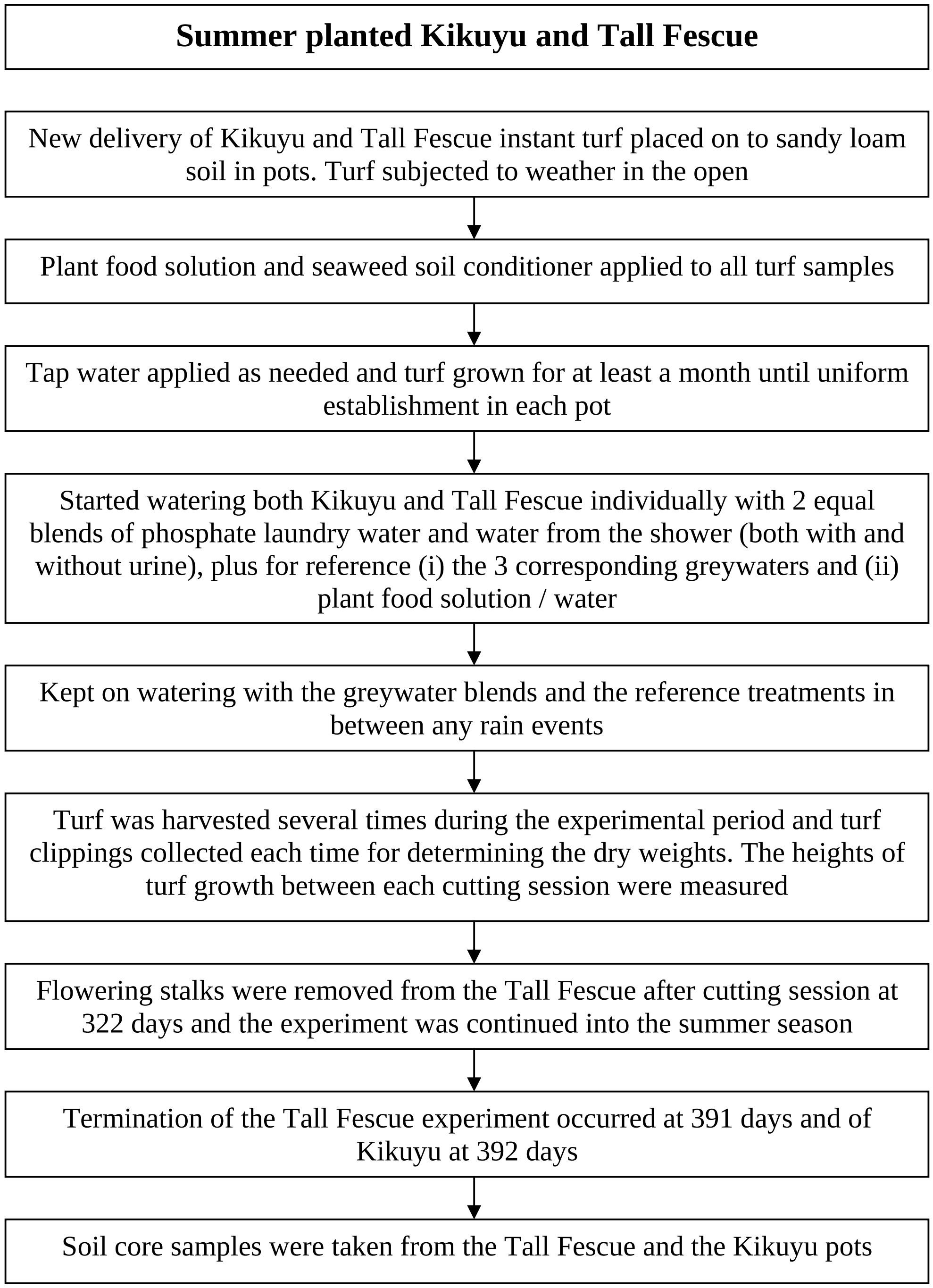

Figure 1.2 – Flow chart showing how the field work study with Summer planted Kikuyu and Tall Fescue turf samples was carried out

Scaly Buttons and Small Vanilla Lilies

Scaly Button and Small Vanilla Lily seedlings planted four to a pot into damp soil mixture composed of equal volumes of sandy loam soil, sieved basaltic soil, and sieved composted eucalyptus mulch. The pots were placed in the open with the plants being subjected to the weather

↓

Exposed soil in the pots was covered with sugar cane mulch, and a plant food solution plus a seaweed soil conditioner were applied to the plants

↓

Water was applied to the plants when needed however the Small Vanilla Lilies died back and all dead stems of the Scaly Buttons were blown off by the wind or were cut off

↓

Addition of 8 different greywaters and 2 reference treatments (i) plant food solution / water, and (ii) water, commenced when new growth was evident in April for the Scaly Buttons and 6 weeks later for the Small Vanilla Lilies

↓

Through the growing period, in between any rain events, the greywaters and reference treatments were applied when needed

↓

Measurements of stem or leaf lengths were taken on 3 occasions throughout the growth period

↓

Experiments were terminated after 36 weeks of growth for the Scaly Buttons, and after 23 weeks for the Small Vanilla Lilies

↓

Final results of plant responses to the greywaters were obtained by (i) counting the flower heads, (ii) determining dry weights of foliage, (iii) determining dry weights of roots, and (iv) measuring longest stem lengths

↓

Soil core samples taken from Scaly Buttons and Small Vanilla Lily pots

Figure 1.3 – Flow chart showing how the field work study with the native flowers Scaly Buttons and Small Vanilla Lilies was carried out

1.5 Structure of the thesis

Chapter I is an introduction to the study. It also details the significance and aims of the research as well as summarising the field work experimental procedures used,

Chapter 2 is a literature review covering:

- Millennium drought, water use restriction, and observed landscaping response by householders.
- Quantity of greywater produced by households.
- Urban heat island effect.
- Phosphate, salts, and urine which may be found in greywater.
- Urine as a plant fertiliser.
- Health risks in using greywater or urine.
- Details about the plants used in the experiments, i.e. the turf species Tall Fescue and Kikuyu, and the native flowers Scaly Buttons and Small Vanilla Lilies.
- How others measured plant growth.
- Summaries of some recently reported studies to grow plants with single source greywaters.

Chapter 3 details the analysis of several types of greywaters for Total P and Total N by modified analytical kit methods, and also details the measurement of the pH of the soils.

Chapter 4 details the method used to grow the two turf species Tall Fescue and Kikuyu with different greywaters and two reference treatments, and also describes the two methods used for determining growth i.e. vacuum method for collecting turf clippings, and measurement of growth height.

Chapters 5 and 6 illustrate, and statistically analyse the growth responses of the turf species Tall Fescue and Kikuyu respectively, both of which were subjected to several types of greywater, and two reference treatments i.e. water and a commercial plant food.

Chapter 7 is a discussion on the Tall Fescue and Kikuyu growth responses as detailed in Chapters 5 and 6 respectively.

Chapter 8 details the method used to grow the native flower species Scaly Buttons and Small Vanilla Lilies with different greywaters and two reference treatments. The four methods of determining growth are; flower head count, foliage dry weight, root dry weight, and stem length measurement.

Chapter 9 illustrates, and statistically analyses the growth responses of the native flowers Scaly Buttons and Small Vanilla Lilies, both of which were subjected to several types of greywater, and two reference treatments i.e. water and a commercial plant food.

Chapter 10 is a discussion on the Scaly Buttons and Small Vanilla Lilies growth responses as detailed in Chapter 9.

Chapter 11 provides recommendations and suggestions for greywater use in domestic and community facility gardens, and provides recommendations for further research.

The Appendix displays in graphical form the monthly rainfall that fell on to the plants during the experimental period, and the monthly total volumes of greywaters that were added per pot during the period. It also provides a complete listing of all the greywater types or treatments that were used in this study.

Chapter 2: Literature Review

2.1 Introduction

The majority of Australian households (93%) have access to mains water (Australian Bureau of Statistics 2010), and households are also major consumers of reservoir sourced water. In the Melbourne urban area 64% of the total consumption during 2013-2014 was used by households, while 25% was accounted for by factories, businesses, schools, hospitals, and parks. The remaining 11% was used for fire fighting, or was lost because of main bursts, leaks, or was unaccounted for (Melbourne Water 2015).

In recent years the availability and use of water has increasingly been an issue of concern in Australia and elsewhere. In south-eastern Australia the Millennium drought (1997-2009) resulted in the annual rainfall being 12.4% below the 20th century mean (SEACI 2011). The drought led to restrictions on the outside use of potable tap water in many Australian locations. In Melbourne the watering of domestic gardens was restricted to specific days and times, and the watering of lawn areas was banned from the time Stage 2 water restrictions came into force during 2006 (Department of Sustainability and Environment 2010). Community centres also faced restrictions with only a select small number of sports grounds being permitted to apply potable water.

During this prolonged drought six large desalination plants were built in Australia (Palmer 2013), so that the major urban centres could be less reliant on rainfall-dependant sources of water, particularly during extended periods of low rainfall.

Some of the projections for the future climate of the Southern Slopes of Australia that are reported in the recently released CSIRO and Bureau of Meteorology commissioned study (Grose 2015) are:

- Increased frequency and duration of extreme droughts.
- Less winter and spring rainfall.
- Reduced soil moisture and run off.

- Higher temperatures.

The restrictions on the watering of lawn areas during the Millennium drought, and the urge to maintain pleasant surroundings, saw the beginning of change in attitudes and behaviour with regards to the design of landscaping to minimise water use (Waterwise Gardens 2013), and also regarding the use of alternate water supplies. In the western suburbs of Melbourne it was not uncommon to see decorative pebbles, gravel, coloured mulches, synthetic turf, concrete, and/or drought resistant plants being used for landscaping over dying lawn areas. On other properties the lawn areas were either left to die off with the hope of regenerating them when conditions were favourable, or attempts were made to maintain some area of green space with greywater or collected rain water.

Watering setups using domestic greywater were often makeshift and very simple e.g. the use of a bucket, or a movable hose or pipe running along the ground and carrying the greywater from the outlet of the bathroom or laundry to the required location. In Western Sydney 28% of survey participants reported using manual bucketing to transfer greywater from the source to the irrigated area, while 72% reported use of an extension pipeline (Pinto & Maheshwari 2008). Many rain water collection tanks that were initially retrofitted in domestic situations were usually too small to provide a regular supply of water during the dry hot summers, and so were probably more suited for providing some extra water for use on gardens in between permitted watering times.

2.2 Quantity of greywater

During the early stages of formulating the project reported here, the work by Christova-Boal, Eden, and McFarlane (1996) was influential in showing that the largest area of need for water in an average household was in the maintenance of gardens, where some 34% of water was used. They suggested that a potentially feasible reuse application for bathroom sourced greywater was in watering of gardens. Around the same time Jeppesen (1996) reported that the use of greywater for lawn and ornamental garden watering, could reduce the average household potable water usage by 30-50%. These estimates however were done before the long Millennium drought, during which time the increasingly severe water use restrictions, and the Victorian Government ‘Target 155’ advertising campaign (Macleod 2009; Savewater 2008), led to changes in the water use habits of the consumers. Target 155 encouraged residents to voluntarily reduce their water usage to no more than 155 litres per person per day (l/p/d). It operated from November 2008 until it was cancelled in 2011 by the new incoming Victorian State Government (ABC News 2011).

The changes in water use habits coupled with the increasing use of water efficient toilets, shower heads, clothes washing machines, rain water tanks, and greywater led to a decrease in per capita potable tap water consumption. Residential water use studies for Yarra Valley Water in Melbourne showed that during the few years between the 2004, and the winter 2010 and summer of 2012, there have been water consumption reductions of 37% in showers, 45% in clothes washers, and 32% in toilets (Arthuraliya, Roberts & Brown 2012; Roberts 2005; Roberts, Arthuraliya & Brown 2011). In an average household the shower had now become the area where the greatest amount of potable water was being used, despite the shower usage reducing from 49 l/p/d in 2004 to 31 l/p/d in 2012.

Although greywater is a limited source of water, it is however regularly available for use throughout dry and water use restriction periods. From the study by Arthuraliya, Roberts & Brown (2012) it can be deduced, that the average person could still produce up to 217 litres of greywater from the shower, and 126 litres from the clothes washer per week. An average family of four people could therefore have available a total of 1370 litres of greywater per week from both sources. It is feasible that considerable savings in potable water usage could also be achieved at sports clubs and elderly citizen’s homes, which often have green space areas that require watering. Community centres usually have the capability of producing greywater in their bathrooms. This bathroom sourced greywater may be environmentally better suited to be utilised on the green space areas, than to be disposed of down a sewer.

2.3 Reducing the urban heat island (UHI) effect

The regular but limited supply of domestic greywater can be useful in maintaining a smallish green space area, and/or in keeping a suite of domestic shrubs and trees alive.

It can also prevent a larger area of browning turf from completely dying off and thus be able to quickly sprout new green growth after the next rain event. Browning of the landscape and lack of water in the environment are not ideal conditions for mitigating excessive high temperatures experienced during heat waves, especially in large urban areas which are susceptible to the urban heat island (UHI) effect (Coutts et al. 2013; Greening Australia 2007). Sustained periods of high temperatures can lead to increases in illness and death, particularly among the elderly, as occurred during the heatwave of January 2009, when Melbourne experienced three consecutive days of maximum temperatures over 43ºC. During that heatwave there were 374 more deaths than was normally expected, which was a 62% increase (Department of Human Services 2009). The UHI effect can be mitigated somewhat by the cooling effect of urban vegetation, which through the processes of evapotranspiration and solar reflection, can reduce the air temperature by 2-8ºC (Fam et al. 2008). To replenish soil moisture and maintain existing vegetation and green spaces Coutts et al. (2013) proposed the harvesting and reuse of stormwater. The use of greywater by households and community facilities however can add to the available water supply for this purpose, or in some areas be the only source of water, especially when there is no significant rainfall for several weeks, and water use restrictions are in force.

2.4 Bulking fillers in laundry water

Greywaters sourced from the typical bathroom or laundry can contain a wide variety of compounds used for personal grooming and cleaning, and for the washing of clothes, bedding, etc. The washing powders designed for use in the laundry have traditionally contained large amounts of bulking fillers such as sodium sulphate (Patterson, R 2009) or sodium chloride (Moulding 2010). However in recent years the volumes of washing powders added to normal wash loads had reduced from approximately 250 ml to 110 ml, and then to 60 ml, as concentrates and later double concentrates replaced the bulkier older style wash powders. The amount of salt that could end up in laundry wash water was therefore being reduced with the development of concentrate washing powders and liquids. The salt concentration of laundry wash water could be further reduced by diluting it with other greywater, tank water if available, or with tap water if water use restrictions did not apply. The strategy of alternate irrigation with greywater and potable water may be a better option to diluting the greywater with potable water. In a glasshouse study of growing silverbeet, the indication was that alternate irrigation was likely to reduce the risk of salinity in soil (Pinto, Maheshwari & Grewal 2009).

2.5 Phosphorus in laundry water

Other ingredients which can add to the sodium content of the wash water include complex phosphorus compounds such as sodium tripolyphosphate (STPP). These are generally used in the more expensive laundry detergents to enhance detergency by softening the water, dispersing clays, and emulsifying oils (Patterson, RA 2004). In the USA the powdered clothes washing detergents contained as much as 60% w/w of STPP or approximately 15% w/w phosphorus (Litke 1999). Sodium tripolyphosphate is also a source of plant nutrient because it eventually hydrolyses to orthophosphate, which can be taken up by plants (Maryland DNR 2005; Wikipedia 2011). Phosphorus is classified as an essential macronutrient which is required for plant growth, and so is one of the three nutrients generally added to soils in fertilisers (Busman L et al. 2009). Some greywater may contain high levels of phosphorus (Ridderstolpe 2004), and ideally this essential plant nutrient could be utilised for the maintenance of amenity green spaces or gardens rather than being discharged down the sewer.

Excess phosphorus and nitrogen ending up in a receiving water body can cause an algal bloom, which in turn can lead to eutrophication of the water (Mylavarapu Rao 2014; NSW Office of Water 2014). It had been reported as early as 2011 that the major manufacturers of laundry detergents in Australia would stop adding phosphates to their products by mid 2014 (Australian Associated Press 2011; Do Something 2011). This action will reduce the amount of phosphorus getting into water bodies from point sources in high population areas, however diffuse sources associated with soil erosion are reported to be the biggest contributors of phosphorus in Australian catchments (Croke 2002).

Madungwe & Sakuringwa (2007) have reported that in Lebanon greywater was a valuable resource of nutrients for encouraging plant growth. On the other hand Jefferson et al. (2004) found that greywater was usually low in plant nutrients, and so the watering of crops with greywater was considered to be mainly a means of recycling water (WHO 2006b). Obviously the composition of greywater can vary according to factors such as the water supply, the source of the greywater, the washing products used, the degree and type of soiling, personal hygiene, and by something as simple as a person urinating while having a shower.

2.6 Urine as plant fertiliser

Urine is considered to be a valuable plant fertiliser that is suitable for use on most non-nitrogen-fixing crops. It contains nutrients in ionic form that are easily taken up by plants, and it provides a fertilising effect that is comparable with ammonium or urea based fertilisers (Mnkeni et al. 2008; WHO 2006b). According to Gunther (2000) the optimum nitrogen to phosphorus ratio (N/P) for nutrient uptake by plants is about 10, whereas the N/P ratio for greywater is 2, and for urine it is 11. The addition of human urine to greywater is therefore likely to increase the nutrient content of the greywater, as well as increasing the likelihood of nutrient uptake by the plants.

Excessive sweating, consumption of large amounts of liquids, and differing diets around the world can affect the quantity of nutrients present in urine according to Jonsson et al. (2004), who estimated that the nitrogen content of urine varied between 3 and 7 g/l. Germer, Addai, & Sarpong (2009) concluded that on average urine contained 7 g/l nitrogen, 1 g/l phosphorus, 2g/l potassium, 1 g/l sulphur, 80 mg/l magnesium, and 200 mg/l calcium. From this they also estimated that about 150 litres of urine, compared to 2 kg of urea, were required to produce 1 kg of nitrogen. Human urine has been used in Sweden and in several other countries to provide the nutrients for growing crops. Jonsson et al.(2004) have determined that the urine from one person can fertilise 300-400m^2 of crop per year, whereas Germer, Addai & Sarpong (2009) state that up to 1000m^2 of crop can be fertilised, depending upon the type of crop. Urine has been applied either undiluted or diluted with water, with the most common dilution being 3:1 (3 parts water to 1 part urine). However dilutions ranging from 1:1 to 10:1 have also been used (Jonsson et al. 2004). Higher dilutions of 10:1 to 15:1 for plants in the growth stage, and 30:1 to 50:1 for pot plants have been recommended by Williams (2006), who also suggested that trees, shrubs, and lawns should cope with undiluted urine.

The simplest method for adding urine to greywater is by urinating while having a shower, and so for the project reported here, it was estimated that domestic shower water could contain from zero to 1% v/v urine. (See section 2.7.1 below for an explanation of how the maximum amount of urine was estimated). At 1% v/v urine the dilution of urine would be about 100:1, at 0.5% v/v urine about 200:1, and at 0.2% v/v urine about 500:1, and so the urine levels used in this work were considerably lower than those that have been normally used for growing crops in other countries. This raised the question as to whether by just urinating in a shower, or collecting urine and adding this back to greywater, a greywater based product that was suitable for frequent use in supplying both the water and nutrient needs of plants, would be a likely outcome. The term *Designer greywater* was used by West (2001) to describe the concept of tailoring the production of greywater with nutrient levels to suit the end use.

2.7 Urine in shower water

In domestic situations small quantities of urine may find their way into greywater sourced from the laundry, but shower water from the bathroom is probably the most likely to occasionally contain a significant quantity of urine. There appears to be no accessible scientific evidence regarding the percentage of people who urinate while showering, but there is plenty of anecdotal information to suggest that the practice does occur. The belief that some people may urinate while showering stems in part from the following:

1. According to a NSW Health publication people often urinate in showers and baths (NSW Health 2000).
2. Traces of urine have sometimes been found in greywater from bathrooms (Eriksson et al. 2002).
3. Brazilian environmental group SOS Mata Atlantica is widely reported to have run TV advertisements encouraging Brazilians to save at least one flush of water a day by urinating in the shower (Fox News 2009).
4. Celebrity singer Kelly Clarkson is reported to admitting that she urinates in the shower (OK Magazine 2009).
5. Urine in greywater is mentioned in publication 07/1380 ‘Greywater Use – Guidelines for residential properties in Canberra’ (ACT Health 2007).

6. Urine in greywater is mentioned in undated online publication accessed in 2015, which answers 12 questions about greywater use (Gladstone Regional Council).
7. There are many on line articles or posted comments referring to people urinating while having a shower, including an on-line poll done by radio station 3AW at the author's request, in which over 70% of respondents said they urinated in the shower.

In community centres such as sports clubs, people are likely to be inhibited to urinate while having a shower, unless the clubs have private showering facilities, or urinating in a shower becomes an encouraged practice on environmental grounds, such as reducing fertiliser use, saving flushing water, or reducing the volume of effluent to be sent to a treatment plant. Urinating in the shower is the simplest method of adding urine to the shower water. However if needed urine could easily be collected separately and added at a later stage. For men the obvious separate source of urine is the urinal. For women, there are toilet bowl systems designed for collection of urine separately from other toilet waste. Collection of urine at sports clubs can be as simple as tapping into the urinal system, or with a bit more difficulty by installing urine separating toilets.

2.7.1 Estimated maximum quantity of urine in shower water

The shower facility that was used for this project had gravity fed hot water, and over several measurements it was determined that about 25 litres of greywater would be produced during a 5 minute shower. The 5 litres per minute shower output fitted in with the most efficient of the Star Rating 3 showers as listed in the Water Efficiency Labelling and Standards (WELS) Scheme. Showers with litre per minute output levels of (>7.5 but <=9.0), (>6.0 but <=7.5), and (>4.5 but <=6.0) are all included under the WELS Star Rating 3 category (Australian Government 2010).

The quantity of urine that a person could discharge during a shower was somewhat difficult to determine. Several sources state that the capacity of a normal bladder is in the range 300-350ml (Encyclopaedia Britannica 2014; Red Orbit 2014; Wikipedia 2014), while others indicate higher capacities i.e. >350ml (Wood D & Walkden M

2012), 300-600ml (Gray 2011), 400-600ml (WebMD 2014), and 450-680ml (Krucik G 2013). On top of that the urge to urinate voluntarily occurs below full capacity (Gray 2011; Krucik G 2013), and female bladders are generally smaller than those of males (Gray 2011; Wikipedia 2014). There is also the uncertainty of how long before having the shower any particular person may have gone to the toilet. Assuming that some people may go and have a shower upon waking from sleep, the writer therefore took self measurements over several mornings. The conclusion was that around 500 ml of urine was generally available first thing in the morning, which would result in a urine content of 2% v/v if it all was discharged into the 25 litres of collected shower water. A more realistic maximum urine level of 1% v/v was however adopted for use on turf samples after considering that:

1. WELS Star Rating 3 showers could output up to 9 litres per minute.
2. Urine output could often be less than 500 ml from part full or smaller bladders.
3. The urge to urinate would usually occur before the bladder was full.
4. People may not have a shower immediately upon waking.
5. Voluntary urine output of men was found to decrease when they aged (Gray 2011).

2.7.2 Possible benefits of using shower water containing urine

The utilisation of urine and shower water or other greywater for growing recreational lawns and gardens at community centres, or at domestic locations should reduce the need for manufactured fertilisers, save toilet flushing water, and also reduce the quantity of effluent to be sent to a sewage plant. Treating less urine-laced effluent should also help to mitigate environmental problems associated with excess nutrients such as eutrophication of receiving waters (Mylavarapu Rao 2014), or hormone and pharmaceutical effects on fish and reptiles (Jonsson et al. 2004). It should also reduce carbon dioxide emissions resulting from manufacturing, treatment, and pumping operations.

As reported above, urine is considered to be a valuable plant fertiliser (Mnkeni et al. 2008; WHO 2006b), which contains 3-7 g/l nitrogen, 1 g/l phosphorus, 2 g/l potassium, 1 g/l Sulphur, and small amounts of magnesium and calcium (Germer, Addai & Sarpong 2009; Jonsson et al. 2004). A reduction in the need for manufactured fertilisers would also save on fertiliser costs and help to conserve resources of phosphorus, an essential nutrient for all life forms (Steen 1998), and of potassium, both of which are reported as possibly being in short supply in about 10 generations (Otterpohl, Grottker & Lange 1997). Other sources estimate that recoverable reserves of phosphorus will last less than 150 years (Gumbo, Savenije & Kelderman 2002), or between 60 and 130 years (Steen 1998), or that the relatively inexpensive phosphorus of today will disappear within 50 years (EcoSanRes. 2005). Because approximately 80% of phosphates produced in the world today are used as fertilisers, it is feasible that a shortage or high prices in the future could cause problems for crop production, especially in developing countries that have not built up soil fertility through many applications of phosphate fertilisers over the years (Steen 1998). Some 25% of mined phosphorus is lost in water environments, landfills, and other sinks, and more than 50% of the phosphorus excreted by humans is in urine (WHO 2006a). It therefore appears that one feasible way of slowing down the loss of phosphorus, is to recycle some of it by using urine to fertilise plants.

2.8 Contamination of greywater and risks to health

In Victoria the recycling of untreated greywater is not subject to legislative control (Department of Human Services 2008) and so it is permitted without Council approval (Better Health Channel 2011), to directly divert untreated greywater during dry periods, or to carry it out by bucket from the laundry or bathroom to the garden surface (EPA Victoria 2008). This is a change from an earlier EPA recommendation that watering with untreated greywater should only be done with subsurface irrigation (EPA Victoria 2001). It appears that low potable water storage levels during the Millennium drought led to a reassessment of how untreated greywater can be safely applied on the garden.

The use of domestic greywater on lawns and gardens is considered to have a low risk of transmitting disease to humans (ACT Health 2007). Greywater may contain pathogens such as bacteria, protozoa, viruses, and helminths (NSW Department of Water and Energy 2008b). Pathogens are primarily added to greywater through contamination with faeces, however it is generally considered that the pathogen content of greywater is low (Ridderstolpe 2004; WHO 2006b). Ridderstolpe also regards greywater as being normally harmless, and argues that the faecal load and therefore the hygiene risk of greywater have been historically overestimated by 100-1000 times in tests undertaken using traditional bacteria indicators. The easily degradable organic compounds in greywater favour the growth of traditional bacteria indicators which may lead to high results, and so Ridderstolpe believes that the hygiene risk of greywater should instead be estimated by the use of chemical biomarkers such as faecal sterols. The theory that use of traditional bacteria indicators may overestimate the hygiene risks of greywater is also supported in other publications (Ottosson 2003; WHO 2006b). Some faecal matter may enter greywater from body washing, and particularly in domestic situations, from the washing of soiled clothing or nappies. Faecal contamination of greywater can be minimised by using disposable nappies and wash cloths, by greater personal hygiene, and by separating faecal soiled items from other items whenever greywater from the laundry is to be used. There is also the option of discharging faeces contaminated greywater to the sewer, but the greywater will be wasted.

2.9 Subsurface irrigation or above ground irrigation

Recommended procedures for watering with greywater appear to be based on how the hygiene risks associated with its use are perceived. Ridderstolpe (2004) suggests simple above ground watering methods, such as the commonly used domestic method with a flexible and manoeuvrable hose, or with a plastic pipe setup containing drilled holes that are not smaller than 6-8 mm. In Victoria untreated laundry or bathroom greywater can be applied directly to the garden (EPA Victoria 2008), whereas in New South Wales subsurface irrigation is still required for directly diverted untreated greywater, although it can be applied to the surface by bucket (NSW Department of Water and Energy 2008a). In South Australia untreated greywater from the bathroom or laundry can be applied to a lawn or garden by bucket, or temporarily by a hose fitted to the outlet of a washing machine (SA Health 2008).

Subsurface irrigation is considered to be a relatively safe method for use with waste water because there is minimal contact between the water and the surface foliage, as was observed by Choi & Suarez-Rey (2004). They conducted reclaimed water trials on Bermuda grass, and found that subsurface irrigation left a dry surface both during and after irrigation. The prudent method of watering lawn and garden areas with greywater at community centres (and arguably also in domestic situations) would be with subsurface irrigation, because it would lessen concerns and objections should urine be added to the greywater. In domestic applications many people will probably continue irrigating with greywater via the simple movable hose method or by bucket, regardless of whether they urinated in the shower, or what the EPA regulations are.

2.10 Health risk in using urine as fertiliser

The potential for urine borne micro-organisms from infected bladders to survive and cause infection is considered to be remote (ACT Health 2007; Gladstone Regional Council). The health risk of using urine as a fertiliser is summarised by the following quote found in a World Health Organisation publication:

> "It can be concluded that pathogens that can be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are not considered to constitute a health risk in the reuse of human urine in temperate climates. **Schistosoma haematobium** is an exception in tropical areas, however, with a low risk of transmission due to its life cycle." (WHO 2006b).

Urine is normally sterile in the bladder of a healthy person but can pick up organisms in the lower part of the urinary tract. The main risk of pathogen transmission in using and handling urine is however based on the amount of faecal cross contamination that can occur during urine collection. To allow for pathogen die off urine is often stored for several months before being used. However if the urine is to used on crops consumed by the household, it would appear from the available literature that it can be used without storage, provided a month is allowed between the last application of urine and the consumption of the crop (Schonning & Stenstrom 2004; WHO 2006a, 2006b). The theory behind this is that disease transmission within a household is more likely to occur because of a lack of hygiene, than because of urine being used as a fertiliser. Fresh urine however should not be used close to surface waters in areas where diseases commonly occur.

Urine is used in several countries as a plant nutrient, and it is generally applied either undiluted or with considerably less dilution than the rates of dilution in the greywaters used for this project (see Section 2.6). The presence of urine was not detectable by appearance or odour in the wastewaters used for the study reported here, even at the maximum concentration of 1% v/v in shower water. It was possible that any faint urine odour may have been concealed by the scents from the body wash products that were used. It is therefore probably feasible to judge the safe use requirements of fresh greywater containing up to 1% v/v urine, as being akin to the safe use requirements of greywater, rather than of neat or partly diluted urine.

2.11 Turf species used in experiments

For this research project it was decided to obtain rolls of two commercially available instant turf species, because instant turf is now commonly used in domestic gardens and community facilities, because it would save establishment time, and because it would provide a high level of consistency of turf biomass and health across the small plots used in the trials. The two species donated by H G Turf Pty Ltd were Tall Fescue *(Festuca arundininacea)* a cool season turf, and Kikuyu *(Pennisetum clandestinum)* a warm season turf. The supplied Tall Fescue was a proprietary blend of two Tall Fescue sub-types of unknown variety.

2.11.1 Tall Fescue

Tall Fescue is a dark green coloured turf and it is described in H G Turf literature as being a hard wearing, low maintenance turf with a deep root system and a broad leaf, and that it is ideal for home lawns and landscaping projects (HG Turf 2006a). Compilation of information sourced from other literature (Harris & Lowien 2007; NSW DPI 2005b; Sanford 2006) describes Tall Fescue as a moderately drought tolerant tussocky perennial grass, which can also tolerate acid and moderately saline conditions, short periods of flooding, and it is suited to a wide range of soils from sandy to heavy clay. The leaves are large and flat 100-600 mm long and 3-15 mm wide. Tall Fescue has poor seeding vigour and so is slow to establish however it responds well to applications of phosphorus, sulphur, and nitrogen. Two types of Tall Fescue are grown in Australia mainly for sheep and cattle grazing:

- Temperate varieties that grow in spring, summer and autumn, and
- Mediterranean varieties that grow well in winter and are summer dormant.

An example of Tall Fescue used in the trials reported here is shown below in Figure 2.1.

Figure 2.1 – Tall Fescue *(Festuca arundinacea)* turf grown in a pot with the aid of Miracle-Gro® All Purpose plant food

2.11.2 Kikuyu

The Kikuyu turf used in this research work is described in H G Turf literature as a self sufficient, drought tolerant lawn that thrives in a range of soils. Its aggressive summer growth with quick spreading runners enables it to be self repairing, and to withstand repeated use (HG Turf 2006b). Other sourced information describes Kikuyu as a perennial creeping sub-tropical grass that forms a dense turf and is tolerant of heavy grazing. It spreads vigorously via rhizomes and leafy branched stolons that root readily from the nodes (Mears 1970; Moore 2006). Kikuyu displays good response to nitrogen fertiliser and irrigation, and it is suited to well-drained very fertile soils (NSW DPI 2005a). However the response of Kikuyu to phosphorus appears to be limited to extremely deficient soils (Mears 1970). During the winter months Kikuyu has a tendency to become dormant and to display yellow colouration, however Mears (1970) states that when nitrogen was applied many workers reported a significantly longer growing season. Kikuyu is a very common turf which can be found growing in parks, nature strips, backyards, and creek banks. Personal observations have indicated that Kikuyu can overtake and swamp an area if left unchecked, even climbing upwards. With adequate mowing and edge trimming, or occasional application of a Glyphosate based herbicide along the edges, it is easily controlled and produces a very robust lawn. An example of Kikuyu used in the trials reported here is shown in Figure 2.2 below.

Figure 2.2 – Kikuyu *(Pennisetum clandestinum)* turf grown in a pot with the aid of Miracle-Gro® All Purpose plant food

2.12 Native flower species used in experiments

The native flower species were Scaly Buttons *(Leptorhynchos squamatus)*, and Small Vanilla Lilies *(Arthropodium minus)*, both of which were purchased from the Iramoo Native Plant Nursery at Victoria University St Albans campus, as individual seedlings in small tapered pots with an opening of 50 mm x 50 mm. The seeds for these plants were sourced from native grassland remnants located on the Keilor-Werribee basalt volcanic plains. Hence the two native flower species used in the trials were indigenous to the area in which the trials were carried out.

2.12.1 Scaly Buttons

Scaly Buttons *(Leptorhynchos squamatus)* are small daisy-like perennial plants with scaly leaflets, and bright yellow flowers of about 15 mm diameters held on long stems. The plants are stated to grow to about 400 mm (Friends of Black Hill and Morialta Inc 2009), but according to another source the height range varies from 100-400 mm (eFloraSA 2007b). Scaly Buttons have a wide distribution across South Australia, Queensland, New South Wales, Victoria and Tasmania. An example of Scaly Buttons in bloom is shown in Figure 2.3 below.

Figure 2.3 – Scaly Buttons *(Leptorhynchos squamatus)* grown in a pot with the aid of Miracle Gro® All Purpose plant food

2.12.2 Small Vanilla Lilies

Small Vanilla Lilies *(Arthropodium minus)* are perennial herbs that flower once per season with pink or purple blooms. The plants have tuberous roots, long and narrow leaves and slender stems. The plants die down in dry weather and sprout again next autumn, and have the ability to regrow from their underground tubers after fire. The height of mature plants is stated to be up to 350 mm (NSW Botanic Gardens Trust), or less than 300 mm (eFloraSA 2007a). The difference is probably due to environmental factors because both height tendencies were observed during the experiments reported here. As with Scaly Buttons, Small Vanilla Lilies are also found in South Australia, Queensland, New South Wales, Victoria, and Tasmania. An example of Small Vanilla Lilies in bloom is shown in Figure 2.4 below.

Figure 2.4 – Small Vanilla Lily *(Arthropodium minus)* grown in a pot with the aid of Miracle Gro® All Purpose plant food

It is not unusual to hear in general conversation or on broadcasted gardening programs that nutrients (e.g. fertilisers) should not be used on Australian native plants. The reason given is that the native plants usually grow in nutrient poor soils. However according to the Australian Native Plants Society (Australia) this belief is a misconception (ANPSA 2009). It has also been reported that some species such as *Proteaceae, Rutaceae, Mimosaceae,* and *Fabacaeae* can suffer from phosphorus toxicity, whereas the great majority of native Australian plants are not sensitive to high phosphorus levels (Leake 1993), and that fertilisers should be used to speed the growth of plants, even phosphorus sensitive ones (Sydney Environmental & Soil Laboratory). The question is “how much” and “how often”, should a fertiliser be applied (ANPSA 2009). Toxicity effects due to phosphorus are said to be lessened by increasing the amount of iron available to the plants, using soils with a high phosphorus fixation such as red clay (Sydney Environmental & Soil Laboratory), or possibly by increasing the nitrogen content without doing this to excess (Leake 1993). Wijessuriya & Hocking (1998) observed in native grassland ecosystems, that when underlying levels of nitrogen and phosphorus are released through plant death resulting from disturbance, high levels of growth can occur in both native plants and weeds.

2.13 Methods used by others to determine plant growth

Determining the dry weight of parts of a plant or of the biomass was found to be a commonly reported method in the literature for obtaining plant growth information (Groeneveld 1998; Lunt & Morgan 1999; Radin et al. 2014). Other methods used include determining the fresh or wet weight, determining the leaf area, measuring the height or length, and counting the number leaves or fruits (Alfiya et al. 2012; Misra, Patel & Baxi 2010; Olayinka & Arinde 2012; Pinto, Maheshwari & Grewal 2009; Salukazana et al. 2006).

To determine dry weights the plants were dried in ovens, however there was a variation between the oven temperatures (55-80°C), and the periods of drying time (24-72 hours) that were reported. Lunt & Morgan (1999) dried grassland biomass for 72 hours at 80°C, while in a different study Morgan & Lunt (1999) dried grassland *Themeda triandra* for 48 hours at 80°C. Wherley (2011) dried grass clippings for 72 hours at 65°C. Groeneveld (1998) dissected grass plants immediately on harvesting, and then measured the roots and individual leaves before drying both for 24 hours at 70°C. Radin et al. (2014) dried Couch grass clippings at 65°C for an unspecified time. Alfiya et al. (2012) did not determine the dry weights of Ryegrass clippings but instead obtained the wet weights within 1-2 hours.

In experiments with vegetables Pinto, Maheshwari & Grewal (2009) obtained the wet weights of silverbeet shoots and roots before drying them for 48 hours at 65°C. Misra, Patel & Baxi (2010) determined the dry weights of tomato roots, branches and stems by drying for 48 hours at 55°C. They also periodically measured the length of a specific branch and a specific leaf, and at harvest determined the leaf area for each replicate pot. Salukazana et al. (2006) stated that they used stem height, number of leaves, fresh and dry weights, and number of fruits to judge the growth of several above and below ground vegetables, however chose to compare only stem height and yield in the report. Olayinka & Arinde (2012) studied the germination and seedling growth in spent engine oil contaminated soil, and used shoot height, number of leaves, stem girth and leaf area to determine plant growth.

2.14 Some recently reported studies using single source greywaters

The study reported in this thesis comprised subjecting two turf species and two native flower species to several types of greywaters that could be sourced from a domestic shower and a washing machine. The plants were grown in pots out in the open and so were subjected to the weather conditions, including the occasional flushing by rain events. The turf species were grown across all four seasons, and the native flowers were grown for one full growth season. The growths of the plants treated with greywaters were compared to the plants treated with a soluble plant fertiliser, and to plants treated with water. Apart from urine being added to some of the greywaters, no commercial plant fertilisers were added during the experimental period to the greywaters or to the soils in the pots.

Since the conclusion of the field work for this study, several other studies as listed below have been reported in which plants were grown with greywaters from, or representing specific sources such as the bathroom or laundry, rather than with mixed greywater. These studies are summarised as follows:

- Misra, Patel & Baxi (2010) conducted a 9 week randomised block design study of growing Grosse Lisse tomatoes in pots in a glasshouse. A spoon of commercially available fertiliser was mixed into the top 5 cm of soil in each pot, and the greywaters were made from a commercially available liquid laundry detergent, and an anionic surfactant. The plants were harvested soon after flowering.

- Pinto, Maheshwari & Grewal (2009) conducted a 60 day completely randomised study of growing silverbeet in pots in a glasshouse. Three gram of chemical fertilizer was added to each pot. The laundry greywater was made from a commercially available concentrate laundry detergent, and the study involved using the greywater, 50% diluted greywater, and alternate use of greywater and potable water.

- Alfiya et al. (2012) conducted a 144 day study over spring and summer of growing Ryegrass (*Lolium perenne*) in sandy loam soil held in planters (pots). The greywater was mainly from showers and washbasins from a building containing 14 flats. Soluble fertiliser solution was added to all of the three irrigation waters used in this study i.e. water, greywater, and treated greywater. The planters were placed outside and no rain occurred during the entire period, however the planters were irrigated with excess water six times during the 144 day experiment

  > "…to wash the soils of accumulated substances…"

  Turf harvesting was conducted four times during the study.

- Radin et al. (2014) grew Couch grass (*Cynodon dactylon L.*) in aquarium tanks that were protected from the rain, in a study to determine "the effects of elements mass balance from turf grass irrigated with laundry and bathtub greywater". Untreated full cycle laundry water, and untreated bathtub water were sourced from the family house and compared against potable water.

In Chapter 3 that follows, there are reports of the analysis of the several types of greywaters used in the experiments reported here for, Total P and Total N. The Total P and Total N levels were obtained by modified analytical kit methods, and the pH results of the soils at the end of the project were determined by a hand held pH meter.

Chapter 3: Analysis of Greywaters

3.1 Introduction

In Chapter 2 it was established that, according to some researchers, although some greywater may contain high levels of phosphorus, greywater is usually considered to be low in plant nutrients and so be mainly a source of water. However, according to other researchers greywater is considered to be a valuable resource of nutrients for encouraging plant growth.

In this project several kinds of domestic greywaters sourced from a laundry and shower were used in experiments to grow turf and native plants. Before applying the various greywaters to the plants, it was important to know what the nutrient compositions of the greywaters were. For the purposes of this study the levels of the main macronutrients nitrogen and phosphorus were determined. It was expected that the levels of N and P in some of the greywaters used were likely to be low, perhaps limiting for growth of plants at some stage. The type of nutrient analysis used for these studies was chosen so as to be able to assess a significant number of samples quickly – based on nutrient sample kits. In the planning stages of this project it was decided that commercially available kits would be used to determine the levels of the nutrients nitrogen and phosphorus.

3.2 Testing for phosphate

The *visocolor® HE* Phosphate (DEV*) kit from MACHERY-NAGEL GmbH & Co. KG in Germany used the Phosphomolybdenum blue method to determine phosphate P (PO_4-P) against a colour comparator disc in the range 0.01-0.25 mg/l P. The kit contained two reagents PO4-1 (powder) and PO_4-2 (liquid), a colour comparator, a measuring spoon, a beaker, and two flat bottom and screw topped glass tubes marked with a line to indicate the required volume of sample. There were problems in using the colour comparator; some of the reference colours could vary between kits, and the ambient lighting, or surroundings such as the blue laboratory bench tops, could cause difficulty in determining accurate results.

The kit however became very reliable and produced a linear absorbance response at 690 nm from 0 to past 1 mg/l P when used with a photometer (Merck SQ118), instead of with the colour comparator disc. The photometer eliminated the uncertainty in determining the readings, and because of the extended range of P levels that could be measured, less repeat tests were needed whenever the dilution of the samples proved to be insufficient. The reliability of the kit when used with a photometer resulted in reagents lasting longer, because it often proved to be sufficient to analyse just one standard near the middle of the linear range, instead of a series of standards. Over a long period the performance of the kit was checked several times on a freshly prepared 0.5 mg/l P standard. The absorbance result never varied by more than 1%.

An extra twenty tubes were purchased so that a supply of clean tubes was always available for doing several tests together. The modified procedure of analysing for PO_4-P with the photometer was:

1. Open the glass tube and rinse with the water sample, and fill up to the mark with the sample.
2. Add 1 level black measuring spoon full of PO_4-1 reagent, close and mix.
3. Open tube and add 15 drops of PO_4-2 reagent, close and mix. Wait 10 minutes.
4. Transfer the test solution to a 10 mm cell, and for a reference blank fill a second cell with the original water sample.
5. Use a photometer to read the absorbance at 690 nm.
6. Calculate the concentration of phosphate P (PO_4-P) by comparing against a calibration curve prepared using standard solutions.

3.3 Testing for nitrate

The Spectroquant® Nitrate Test kit 1.14773.0001 from Merck KGaA (64271 Darmstadt, Germany) was initially used for nitrate N (NO_3-N) analysis. The kit contained two reagents NO_3-1 (powder) and NO_3-2 (liquid), and according to the brochure the test method was described as follows: “In concentrated sulphuric acid nitrate ions react with a benzoic acid derivative to form a red nitro compound that is determined photometrically”. With 10 mm cells the measuring range was quoted as 0.5 - 20 mg/l NO_3-N. This kit did not give as reliable results as the phosphate test kit, with results sometimes varying by 25 - 40%, and so a lot of repeat tests and extra tests on standards had to be done. The testing with this method tended to be somewhat frustrating, and the reagents were quickly used up.

From Material Safety Data information it was determined that reagent NO_3-1 was likely to be 3,5-Dihydroxy benzoic acid so this was tried as a replacement when the kit reagents were getting low. There were also several Winchester bottles of BDH 98.07% Analytical grade concentrated sulphuric acid in stock at the laboratory. The 98.07% concentrated sulphuric acid, as well as sulphuric acid diluted to approximately 90% and 97% concentrations were compared against the NO_3-2 reagent. After several evaluations it was determined that 3,5-Dihydroxy benzoic acid was a satisfactory replacement for reagent NO_3-1, and that only the 98.07% sulphuric acid gave similar results to that given by reagent NO_3-2. The two diluted acids produced lighter colour development and lower absorbance readings. The BDH 98.07% concentrated sulphuric acid and 3,5-Dihydroxy benzoic acid were used for all future NO_3-N analysis.

The use of the 10 mm cells for analysing was not ideal from time or safety points of view. The high viscosity concentrated sulphuric acid based reacted samples did not drain very well from the cells walls, and so care had to be taken to ensure that the cell was adequately rinsed out with the next reacted sample, and that there was enough left over of the next sample to fill the cell. Fortunately the SQ118 photometer could also read the absorbance of a sample held in a 16 mm round glass tube. Tests had shown that although the absorbance readings with the 16 mm glass tubes were higher than with the 10 mm cells, the final results, after comparing against standards tested in similar ways, were basically the same. Several boxes of 16 mm round glass tubes and plenty of replacement screw caps were therefore purchased, and all future absorbance readings were performed using these tubes. Leaving the samples in the closed tubes increased the safety of handling a concentrated acid based solution, and when needed permitted absorbance readings to be taken on the same sample at several time intervals of colour development.

The modified procedure for analysing for nitrate nitrogen (NO_3-N) was based on the Merck procedure, and was as follows:

1. Place 1 micro spoon of 3,5-Dihydroxy benzoic acid into a clean and dry 16 mm screw top tube.
2. Pipette into the tube 5.0 ml of concentrated sulphuric acid. Close the cap securely.
3. Cover the cap with a folded piece of paper towel and securely hold the paper and the tube near the cap.
4. Shake vigorously for 1 minute.
5. Very slowly pipette 1.5 ml of the sample down the walls. To prepare a reference blank use 1.5 ml of deionised water instead.
6. Close the tube, cover the cap with a folded piece of paper towel, and mix by shaking for about 1 minute. (Caution: the tube will get hot).
7. Set aside for 45 minutes for colour development.
8. Place the tube in the Merck SQ118 photometer and measure the absorbance at 525 nm.
9. Calculate the concentration of NO_3-N by comparing against a calibration curve prepared using standard solutions.

Occasionally a tube did not seal well and some acidic mixture could leak out while the tube was being shaken. The folded paper towel soaked up any leaked material, and also made it easier to hold the tube when it became hot. Any sample that leaked was discarded and the test repeated.

The colour development time was longer than the 10 minutes suggested for use with the nitrate test kit. It was found that full colour development could take about 20 minutes for samples with low nitrate content, while samples with higher nitrate content could take around 45 minutes. It also took 4-5 minutes to carry out the reaction procedure on each sample, and so allowing about 45 minutes enabled 8 or 9 samples plus a blank to be prepared before the first photometer reading had to be taken.

3.4 Digestion of samples

The two kits could only test for simple, reactive nitrate and phosphate and so to test the samples for the required total nitrogen (Total N) and total phosphorus (Total P), the samples had to be digested to convert the various nitrogen or phosphorus containing compounds to the reactive nitrate or phosphate forms.

3.4.1 Digestion procedure for testing of Total P

The procedure was adapted from method 4500 PB in *Standard Methods for the examination of Water and Wastewater* (Franson et al. 2005) and was as follows:

1. Measure out 50 ml of sample (or 50 ml of an accurately diluted sample) into a conical flask. For a reference blank use 50 ml of deionised water.
2. Add 1 drop of phenolphthalein indicator.
3. Discharge any red colour by adding drops of 30% Sulphuric Acid.
4. Add an extra 1 ml of 30% Sulphuric Acid.
5. Add 0.5 g of Potassium persulphate.
6. Boil gently on a hot plate for 30 to 40 minutes, or until volume goes down to 10 ml.
7. Cool. Then dilute to 30 ml.
8. Add 1 drop of phenolphthalein indicator.
9. Neutralize to a faint pink colour with 1N Sodium Hydroxide.
10. Make up to 100 ml in a measuring cylinder. (Note that the volume of the sample has now doubled).
11. Do not filter if any precipitate is formed.
12. Analyse for phosphate phosphorus (PO_4-P) as described above in Section 3.2 Testing for phosphate.

3.4.2 Digestion procedure for testing of Total N

This Persulphate digestion procedure was adapted from method 4500 NC (Franson et al. 2005). With this method of digestion the organic and inorganic nitrogen compounds are converted to nitrates by alkaline oxidation at 100-110ºC. Samples preserved with acid however cannot be analysed. The procedure used for the digestion was:

1. Prepare fresh digestion reagent by adding 1.0005 g of $K_2S_2O_8$ (potassium persulphate) and 10.0 ml of 15 g/l NaOH to a 50 ml standard flask, top up with water, cap, and shake.
2. Add 10.0 ml of sample or standard (or a portion diluted to 10.0 ml) to a screw top culture tube (20 mm OD x 150 mm long). For a reference blank use 10.0 ml of deionised water
3. Add 5.0 ml of the freshly prepared digestion reagent.
4. Close the tube tightly with a polypropylene wadless screw cap. Mix by inverting twice.
5. Heat for 30 minutes in a pressure cooker.
6. Slowly cool to room temperature.
7. Analyse for nitrate nitrogen (NO_3-N) as described above in Section 3.3 Testing for nitrate.

In the 4500 NC method the instruction is to make up 1 litre of fresh digestion reagent just before use. However only 50 or 100 ml of digestion reagent was ever required each time the digestion of samples was performed. It was not easy to accurately weigh out 0.06 g or 0.12 g of solid pearl NaOH to make up 50 or 100 ml of digestion reagent, so the procedure was altered to use 10.0 ml of 15 g/l NaOH solution instead.

To hold the culture tubes upright and to prevent the tubes from touching the base of the pressure cooker, a plastic rack which could hold up to 20 tubes was constructed. The plastic rack was cut from a larger rack that was known to resist the cooking temperature. A soldering iron was used to weld a couple of plastic spacer legs on the cut side of the rack.

3.4.3 Digestion of Sodium Tripolyphosphate (STPP)

Sodium Tripolyphosphate ($Na_5O_{10}P_3$) was used to determine the efficiency of the digestion process for converting complex phosphate compounds to the simple reactive phosphate. Solutions of 10 mg/l P, 20 mg/l P, and 40 mg/l P were prepared from STPP and received total dilutions of 20, 40, and 80 fold respectively during the digestion and analysis process.

The % Recovery (or conversion) results are shown in Table 3.1 from which it can be seen that the Total P digestion process is efficient in converting Sodium Tripolyphosphate to reactive phosphate, with an average recovery result of 98.5%. A sample of phosphate based laundry water had extra STPP added to it and the digestion process gave an average recovery result of 96.8%. The undigested standard of STPP had only 3.4% conversion after 15 minutes of colour development time. However the colour development kept on slowly increasing for a long time. The conclusion is that the Total P digestion process is very efficient in converting STPP to phosphate. The undigested 10 mg/l P (PO_4-P) sample was made from K_2HPO_4 which confirmed the accuracy of the test for phosphate with a result of 98.8%.

Table 3.1 – Total P results for Sodium Tripolyphosphate (STPP) solutions

Digested Sample	mg/l P	% Recovery
10 mg/l P (STPP)	9.84	98.4
20 mg/l P (STPP)	19.84	99.2
40 mg/l P (STPP)	39.2	98.0
Average		**98.5**
Digested Laundry Water		
Laundry Water (LW)	16.1	-
LW plus 20 mg/l P (STPP) spike	35.2	95.5
LW plus 20 mg/l P (STPP) spike	35.7	98.0
Average		**96.8**
Undigested Sample		
20 mg/l P (STPP)	0.68	3.4
10 mg/l P (PO_4-P)	9.88	98.8

3.4.4 Digestion of Nicotinic Acid (NA)

The kit method could only test for simple reactive nitrate. To determine the total nitrogen (Total N) contents of the greywaters, complex nitrogen compounds such as proteins and amino acids that may have been present, needed to be digested to reactive nitrate.

Nicotinic acid ($C_6H_5NO_2$) was used to determine the efficiency of the persulphate digestion process for converting various nitrogen containing compounds to the simple reactive nitrate. Nicotinic acid was purchased after experiencing varying digestion results with L-Glutamic acid. The problem experienced with persulphate digestion of L-Glutamic acid standards, and the suspected cause of the problem is detailed is Section 3.4.5 below.

From Table 3.2 it can be seen that the persulphate digestion process is efficient in converting Nicotinic acid to reactive nitrate with an average recovery result of 98.4%. The undigested sample of Nicotinic acid 2 mg/l N (NA) shows that without digestion there is no detectable nitrate present, and the two undigested samples containing nitrate N (NO_3-N) show that the test for nitrate is acceptable, however a recovery in the vicinity 96.6% does not always occur. The fact that a few of the recovery results for Nicotinic acid are above 100% is of no concern. The results are balanced by some of those below 100%, and it just indicates that there is an error range in determining the Total N with this procedure.

Table 3.2 – Total N results for Nicotinic Acid (NA) solutions

Digested Sample	mg/l N	Recovery %	Digested Sample	mg/l N	Recovery %
2.5 mg/l N (NA)	2.37	94.8	2 mg/l N (NA)	1.84	92
2.5 mg/l N (NA)	2.35	94	2 mg/l N (NA)	2.14	107
2.5 mg/l N (NA)	2.55	102	2 mg/l N (NA)	2.0	100
3.75 mg/l N (NA)	3.9	104	2 mg/l N (NA)	2.0	100
3.75 mg/l N (NA)	3.7	98.7	5 mg/l N (NA)	4.8	96
5 mg/l N (NA)	4.78	95.6	5 mg/l N (NA)	5.0	100
5 mg/l N (NA)	4.79	95.8			
5 mg/l N (NA)	4.85	97			
			Average		**98.4**
Undigested Sample					
2 mg/l N (NA)	0	0			
2.9 mg/l NO_3-N	2.85	98.3			
5 mg/l NO_3-N	4.74	94.8			
Average		**96.6**			

3.4.5 Problem experienced with persulphate digestion method

In the 4500 NC method for persulphate digestion (Franson et al. 2005) the recommendation is to analyse the digested samples for nitrate with a cadmium reduction method. Standard solutions based on L-Glutamic acid and Potassium nitrate (KNO_3) are suggested and these are preserved by adding 2 ml of Chloroform per litre of standard solution.

For this project the persulphate digested samples were analysed for nitrate by a modified procedure based on a Merck kit. It was found that persulphate digested standards based on KNO_3 and on L-Glutamic acid sometimes produced higher than expected results, which could vary from 20% higher to 200-300% higher. Standards that produced higher results also tended to develop a different colour. The expected crimson red colour was often affected by a yellow colouration to varying degrees, with some samples producing a light orange colour.

The pressure cooker originally used had dial up low and high pressure settings and hence cooking could be done at unknown low and high temperatures above 100ºC. The problem mainly occurred when using the high pressure setting. Fortunately the pressure cooker broke down and was replaced with an Arcosteel 8 litre cooker that cooked only on one unknown pressure and temperature. The results with this new cooker were worse.

The L-Glutamic acid came from another campus of Victoria University and was not in its original container, and so it was decided to purchase Nicotinic acid to use as a standard instead, in case L-Glutamic acid caused the problem. There was uncertainty about whether standards made from Nicotinic acid should be preserved with Chloroform or not, and so no Chloroform was added. The Nicotinic acid based standard solutions that underwent persulphate digestion always produced the expected red crimson colours, and gave good recovery results as can be seen in Table 3.2 above. Nicotinic acid was therefore adopted as the standard for use with the persulphate digestion process, and the L-Glutamic acid was no longer used even without Chloroform because of the uncertainty of its origin. No yellowing of developed colour was ever observed when analysing undigested standards based on KNO_3.

In summary it is suspected that persulphate digestion of standard nitrogen solutions which contain Chloroform as preservative, may produce compounds that interfere with the kit method of analysis for nitrate, a method which relies on a crimson red colour development. It is also suspected that digestion at a higher temperature may increase the problem. Time did not permit an investigation as to the validity of these suspicions.

3.5 Analysis of greywaters

The greywaters, water, and the Miracle-Gro® solution used to grow the Turf and Native Flower samples are often referred to as 'treatments' in this thesis. A code system is used throughout the thesis to identify the treatments. The codes, as shown in bold characters in the list below, are also used to refer to the various types of greywater analyses reported in this section:

1. **Water** – Melbourne tap water.
2. **M/Gro** – Miracle-Gro® solution. Made by dissolving a 15ml level scoop of Miracle Gro® All Purpose Plant Food per 4 litres of tap water.
3. **CPTW** – Cold Power Total Wash. The total collected laundry greywater (wash and rinse cycles) when using Cold Power® Advanced concentrate laundry powder.
4. **CPDR** – Cold Power Deep Rinse. The deep rinse only greywater collected when using Cold Power® Advanced concentrate laundry powder. Excludes the waters from the wash and spray rinse cycles of a washing machine.
5. **ECTW** – Earth Choice Total Wash. The total collected laundry greywater (wash and rinse cycles) when using Earth Choice® laundry liquid.
6. **ECDR** – Earth Choice Deep Rinse. The deep rinse only greywater collected when using Earth Choice® laundry liquid. Excludes the waters from the wash and spray rinse cycles of a washing machine.
7. **Shower** – Total collected greywater from bathroom shower when washing body. Only liquid body wash and shampoo products were used and no solid soaps.

8. **SHU** – Greywater from bathroom shower as above, with 1% v/v human urine added. Used only on the Turfs.
9. **SHU/2** – Greywater from bathroom shower with 0.5% v/v human urine added. Used only on the Native Flowers.
10. **SHU/5** – Greywater from bathroom shower with 0.2% v/v human urine added.
11. **CPTW SHU (or CPTW & SHU)** – An equal volume blend of CPTW and SHU greywaters. Used only on the Turf samples.
12. **CPTW Shower (or CPTW & Shower)** – An equal volume blend of CPTW and Shower greywaters.
13. **CPTWU** – Cold Power Total Wash plus Urine (initially 1% v/v and later reduced to 0.5% v/v). Added in the latter stage of the experiment to Spring set of CPTW treated turf samples in an attempt to encourage growth.

3.5.1 Total P results of greywaters

The results in Figure 3.1 show that of the greywaters used in this project:

- The highest levels of phosphorus compounds were found in greywaters containing the Cold Power® based total wash water CPTW or blends with CPTW.
- The deep rinse CPDR contained 44 or more times less phosphorus than the relevant total wash CPTW.
- Urine made a significant addition to the phosphorus content of greywaters.
- ECTW had a relatively higher phosphorus result on 7/04/08, which may have been due to the type of items that were laundered.
- The lowest phosphorus containing greywaters were ECDR, Shower water, CPDR and ECTW.
- The phosphorus contents of all the greywaters were very small, when compared against the phosphorus content of the Miracle Gro® solution.
- The general levels and trends in P content of the various greywater samples reported above was consistent across three sampling times.

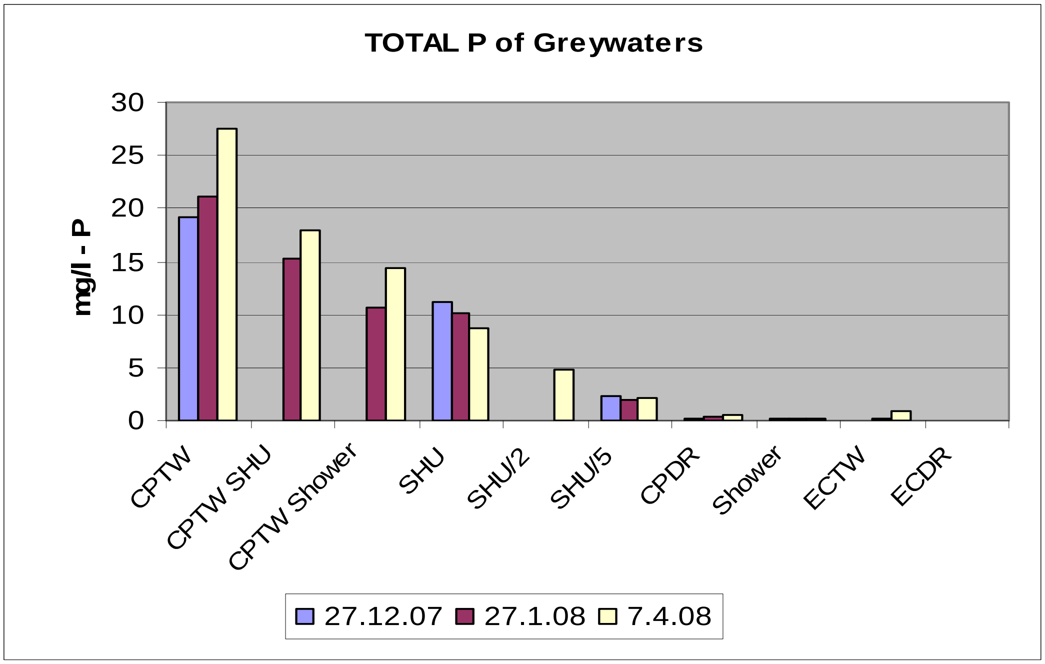

Figure 3.1 – Average Total P results (mg/l P) of greywaters sampled on three occasions.

The Total P content of Miracle Gro® solution is not included in Figure 3.1 because at 452 mg/l P, it is far in excess of the Total P contents of any of the greywater samples.

3.5.2 Total N results of greywaters

From Figure 3.2 it can be seen that:

- The major source of nitrogen compounds found in the greywaters used during this project was due to the added urine.
- The deep rinse waters CPDR and ECDR contained the least amount of nitrogen compounds, with ECTW not far behind them, and Shower alone slightly above these.
- The urine containing greywaters especially SHU and SHU/2 have significant amounts of nitrogen compounds when compared with Miracle Gro® solution.
- Laundry waters including CPTW had relatively low amounts of nitrogen compounds.
- These trends in N content were consistent across three sampling times.

It appears likely that with frequent application of greywaters during dry hot weather, the total amount of nitrogen compounds that SHU could add to turf samples, could be greater than that added with a single Miracle Gro® application every fortnight.

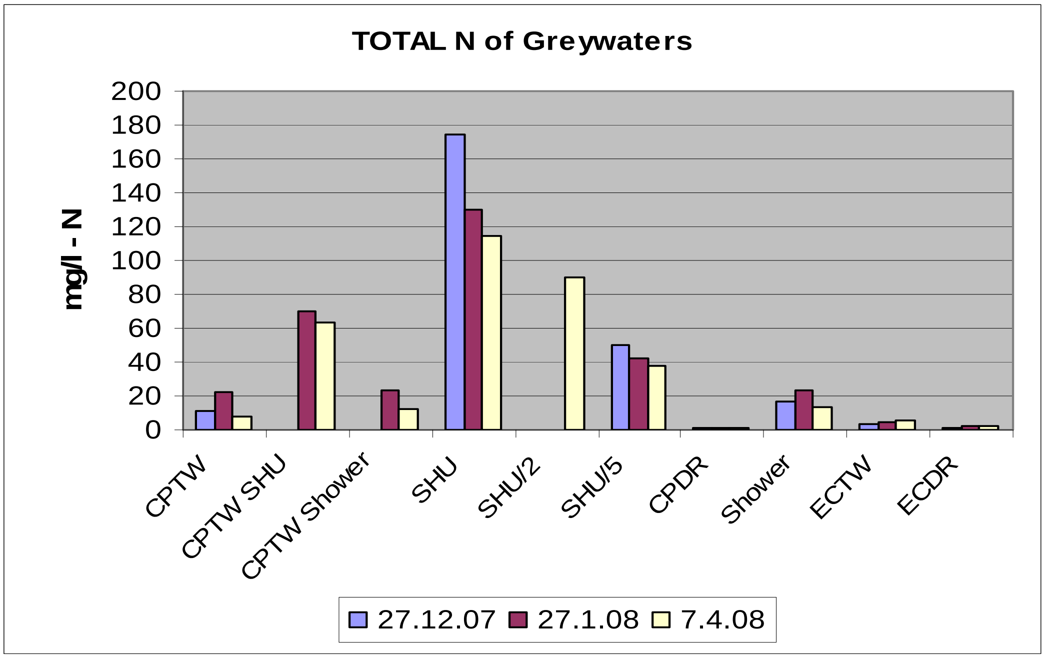

Figure 3.2 – Average Total N results (mg/l N) of greywaters sampled on three occasions.

The Total N content of Miracle-Gro® solution is not included in Figure 3.2 because at 474 mg/l N, it is far in excess of the Total N contents of several of the greywater samples, and it is significantly greater than the next highest result of 175 mg/l N for SHU greywater sampled on 27 December 2007.

3.5.3 pH results of greywaters

Figure 3.3 shows that:

- The phosphate based Cold Power® laundry powder produced greywaters with the highest pH results (CPTW and blends of CPTW).
- The total wash CPTW exceeded a pH of 10 on two out of three occasions.
- The CPTW blends had higher pH values than the other greywaters.

- Blends of Shower water plus urine (SHU, SHU/2, and SHU/5) all produced pH results between 6 and 7.
- The deep rinse waters CPDR and ECDR had pH results of just above 7.
- Miracle-Gro® solution had by far the lowest pH at 4.9.
- These trends in pH were consistent across the three different sampling times.

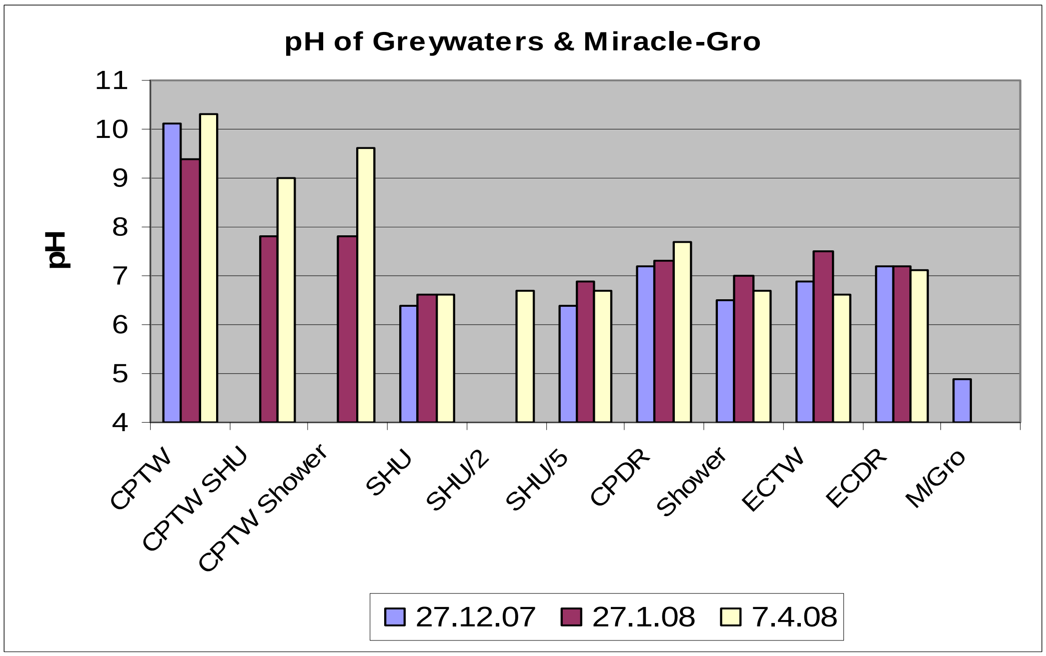

Figure 3.3 – Average pH results of greywaters sampled on three occasions plus of Miracle-Gro® solution

3.5.4 Effect of greywaters on pH of soils

The soil used for growing the turf samples was sandy loam top soil. However the soil used for growing the native flowers was an equal parts mixture of sandy loam top soil, sieved soil from grasslands near Iramoo Plant Nursery, and sieved partly composted eucalyptus mulch. Different soil types were used for the turf and the native flower experiments because the instant turf would normally be laid out on a bed of sandy loam, and the native flowers would normally be planted in the garden sections. The primary purpose of the experiments was to compare the growth of each species under the various wastewater treatments, not comparisons across species, so use of different soils for wildflower species and turf species is of no consequence for the results of these experiments. The turf soils were sampled near the end of the project with a minimum of four days being maintained between the application of greywater and taking a soil core sample. The native flower soils were sampled at the end of the experiment when the plants were being harvested. The project was conducted with five replicates for each type of plant and treatment applied to the samples. To minimise the number of samples to be tested, equal weights of each of the five replicates were mixed together to form composite soil samples.

The pH tests on the composite soil samples were conducted as per the method used previously by Wijesuriya (1999) at Victoria University. The pH measurements were done with hand held Oakton pH Testr10, and the instrument was calibrated with buffers of pH 4.00, 7.00, and 10.00. The method of determining the pH was:

1. Accurately weigh out 5 gram of air dried soil into a 50 ml screw cap bottle.
2. Add 25 ml of deionised water and close the cap.
3. Shake on a mechanical shaker for 1 hour.
4. Allow the suspension to settle for 30 minutes.
5. Measure pH.

Figure 3.4 below shows the pH results of the sandy loam soils in which Kikuyu and Tall Fescue turf samples were grown. It can be seen that:

- Higher soil pH results occurred with greywaters CPTW or blends of CPTW.
- The higher soil pH results corresponded with the higher pH results of the greywaters CPTW or blends of CPTW.
- The highest soil pH results were recorded with CPTWU which was a blend of CPTW and urine, and which was used towards the latter part of the project.
- Shower water and blends of Shower water plus urine (SHU and SHU/5) however produced similar pH results in turf soil to that produced by water.
- The lowest soil pH values resulted from the Miracle Gro® and SHU treatments applied to Tall Fescue turf.

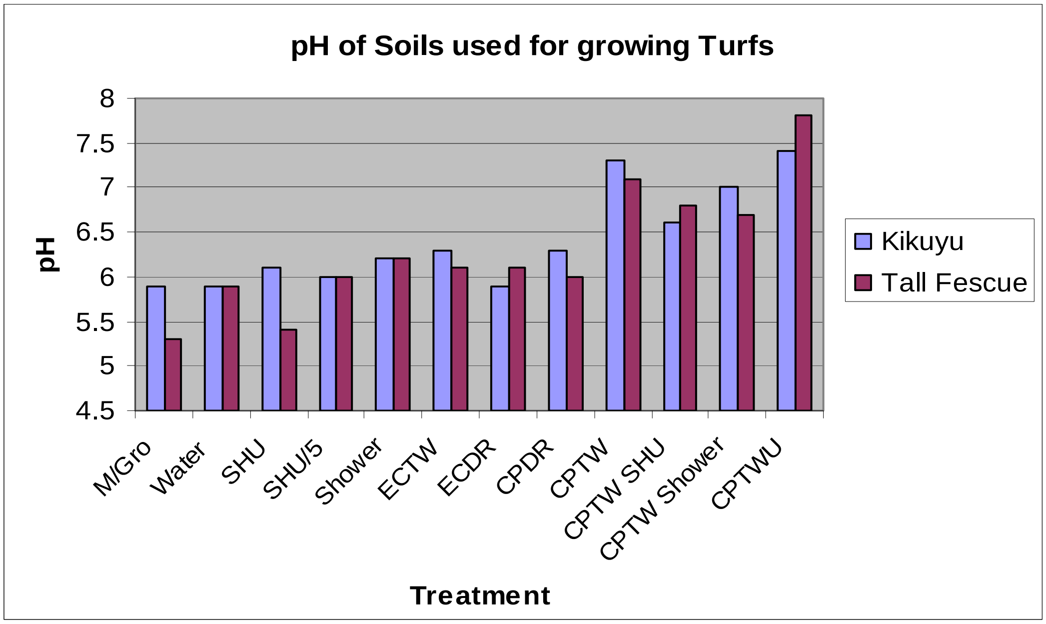

Figure 3.4 – pH results of soils used to grow Kikuyu and Tall Fescue turf samples with several watering treatments

Figure 3.5 below shows the pH results of the soils in which the native flowers were grown. It can be seen that:

- The pH results of the soils were all similar, however the soils in which the Small Vanilla Lilies grew had slightly higher pH (0 to 0.5 pH units), than the soils in which the Scaly Buttons grew.
- The lowest pH of 6.2 was recorded by Scaly Button soil treated with Miracle Gro® solution.
- The highest pH of 6.9 was recorded by Small Vanilla Lily soil treated with CPTW.

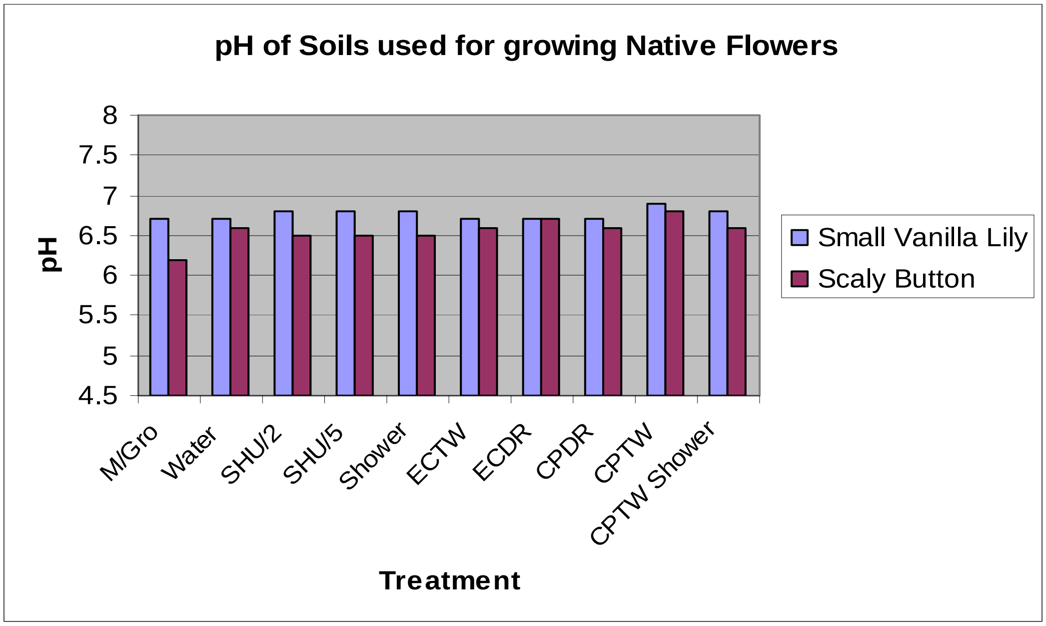

Figure 3.5 – pH results of soils used to grow Small Vanilla Lily and Scaly Button native flower samples with several watering treatments

3.6 Discussion

The analytical results show that the laundry greywater CPTW, and to a lesser extent blends of CPTW, contained the largest levels of Total P phosphate, and these greywaters also recorded the highest pH values. CPTW however contained relatively low Total N nitrate. Greywaters with added urine, especially SHU from the bathroom, contained relatively high amounts of both phosphate and nitrate, while the deep rinse laundry greywaters, Shower water, and ECTW did not contain significant levels of phosphate or nitrate. The nutrients in urine are generally available for plant uptake however the complex phosphate in CPTW based greywater needs to be hydrolysed to a reactive form to become a nutrient. What is unknown is how much of the complex phosphate may exit the pots before being hydrolysed to orthophosphate, and so not be available as a plant nutrient.

Tests for pH on the soils used to grow the turf samples show that for both types of turf, the samples treated with CPTW and with CPTW blends recorded the highest (most alkaline) readings. The soils in which the native flowers were grown recorded similar pH values across the treatment range, with the Small Vanilla Lily samples generally producing slightly higher soil pH values. There was no significant increase in soil pH resulting from CPTW based treatments on native flower samples. The turf and native flower samples were however grown in different soils, and the timing of soil sampling differed for the turf and native flower samples. Changes in pH can affect the levels of nutrients available for plant uptake and hence plant growth. These differences are taken into account in discussion of the results of plant growth under different wastewater treatments.

The next Chapter 4 outlines the methodology used to conduct the experiment of growing Tall Fescue and Kikuyu turf varieties with several specific greywaters sourced from a bathroom and laundry.

Chapter 4: Greywater experiments on two types of turf – Experimental Methods

4.1 Introduction

The two varieties of turf used for this experiment were Tall Fescue *(Festuca arundinacea)* and Kikuyu *(Pennisetum clandestinum),* both of which were donated by H G Turf Pty Ltd as rolls of instant turf. These two varieties were selected because Tall Fescue was described as a popular cool season turf and Kikuyu as a popular warm season turf. For further information about Tall Fescue and Kikuyu grasses see Chapter 2 (Sections 2.11.1 and 2.11.2).

4.2 Pots, soil, and planting the turf

4.2.1 Pots

The turf growing experiment was conducted in pots constructed from 12 litre square buckets sold by the hardware chain Bunnings Warehouse. The slightly tapered buckets were 230 mm high with an opening at the top of approximately 250 mm x 250 mm, thus being capable of containing a reasonable sized turf sample. Eight drainage holes (19 mm diameter) were drilled into the sides of the pots near the bottom. Square buckets were chosen to minimise the wastage resulting in cutting turf sections from the rolls of instant turf.

4.2.2 Soil

Each pot was filled with sandy loam top soil purchased from a local supplier. The damp soil was compacted by lightly tapping it down with a brick and filling of the pot was halted when the soil was approximately 40 mm from the top.

4.2.3 Planting the turf and initial care

It took a few days to plant the turf into the pots and so to prevent the rolls of instant turf from drying out they were rolled out onto a semi shaded recently mown lawn area, and kept watered until used in the pots. The rolls of turf were cut into pot sized shapes with a sharp knife while still on the ground, and then placed on top of the soil in the pots. Any small gaps were filled with sandy loam soil. The turf was allowed to establish over approximately eight weeks by regularly watering, and conditioning once with Seasol® seaweed concentrate, and feeding twice with water soluble Miracle-Gro® All Purpose plant food. No feeding of the turf samples was done for a month prior to commencing the addition of greywaters.

4.2.4 Setback with Kikuyu

The first set of Kikuyu and Tall Fescue samples were obtained in late August 2007 and were allowed to establish in pots until ready to be treated with greywaters. Experiments with the Tall Fescue commenced on 9 November 2007, however, the experiments with the Kikuyu had to be delayed to 16 January 2008 because of unforeseen Rye grass contamination, which had to be removed by hand. This problem, and how it was resolved, unfolded as follows:

Soon after the Kikuyu was placed in the pots it was thought to be growing exceptionally well for that time of the year, however, suspicions were not aroused until stalks began to grow. On discussing the problem with a representative from HG Turf it was concluded that the supplied Kikuyu was meant for winter sale and so was over sown with Rye grass, which is used to compensate for the tendency of Kikuyu to yellow and become dormant over winter. The initial thought was to use the Kikuyu plus Rye grass for the experiments but it was obvious that the Rye grass was not uniformly represented in each pot. This variation between pots was an unacceptable situation, because it would have an adverse affect on determining turf growth differences associated with the greywaters used. Over several days all noticeable Rye grass was carefully removed from each Kikuyu pot by hand, and the remaining self repairing Kikuyu was again treated with Miracle-Gro® All Purpose plant food to help consolidate uniform establishment across the pots.

4.2.5 Spring and Summer planted turf

In early November 2007 a second set of Tall Fescue and Kikuyu turf was obtained from HG Turf, primarily for the purpose of evaluating blends of laundry and shower based greywaters. The Kikuyu from the second set however had not been over sown with Rye grass, and so because of the possibility that this Kikuyu may behave differently to the Kikuyu from the first set, the two sets of Kikuyu were not combined together for the experiments. It was therefore decided to conduct two separate experiments, with evaluation of the two blends of greywaters from the laundry and shower being conducted on the second set of turf samples. This required that some of the treatments applied to the first set of turf samples be duplicated on the second set. The first set of turf which arrived late August 2007 was designated as the Spring planted set, while the turf which arrived early November 2007 was designated as the Summer planted set. Although the Tall Fescue did not present the same problem as the Kikuyu, the two sets of Tall Fescue were also kept separate. Simple, abbreviated labelling is used throughout this thesis to distinguish between the four sets of turf, as follows:

- Kikuyu (Spring) – Kikuyu from the Spring planted set.
- Kikuyu (Summer) – Kikuyu from the Summer planted set.
- Tall Fescue (Spring) – Tall Fescue from the Spring planted set.
- Tall Fescue (Summer) – Tall Fescue from the Summer planted set.

4.3 Watering of the turf samples

Initially sub-surface watering was attempted by using four plastic watering spikes to which plastic funnels, which were cut from the tops of soft drink bottles, were attached. Experiments with just sandy loam soil in a pot, showed that one spike in the centre was not sufficient to adequately water the soil out towards the edges. However the use of four watering spikes placed about 70 mm in from each corner watered the soil satisfactorily, although the setup with attached funnels tended to selectively block the sunlight. Replacement and removal of the spike setup for each watering session enlarged the holes in the soil, and so the greywater tended to break on to the surface. The procedure also took far too long to water all the plants. Seeing that the greywater tended to break on to the surface, it was decided to just sprinkle the greywater directly on to the plants. A sprinkling cup was constructed by drilling holes into the base of a 600 ml plastic cup. Usually 300 ml of greywater was tossed into the sprinkling cup, which was then moved quickly over the entire turf surface in the pot. This watering procedure proved to be a considerably faster method than by using the spike setup.

4.3.1 Rain included in experiment

The waters used for the experimental growing of the turf samples in this thesis are often referred to as ‘treatments’, and these waters include several greywaters, Miracle-Gro® solution, and tap water. Refer to Chapter 3 (Section 3.5), or to Appendix C for a list describing the origins, compositions, and the identifying codes of the waters used on the turf or on the native flower specimens. Rain was part of the turf watering process because rain would generally not be excluded from domestic or community centre situations, where greywaters were used to grow turf. A rain gauge was used to determine whether there was sufficient rain to forego further watering with greywaters for a day or more. Quite often the appearance of water on the turf in the morning was sufficient to judge whether rain had fallen overnight, but the rain gauge often revealed that the total fall was 0.5 mm or less, which was insufficient (during the extended drought). See Appendix A for the measured monthly rainfall over the experimental area during the period 1/11/07 to 15/3/09, when seven months had from zero to 15 mm of rain. The total rainfall for 2008 over the experimental area was 346.2 mm, which was 62.2% of the Mean annual rainfall of 556.9 mm during the period 1971-2000, as measured 8 km away at Melbourne Airport (Bureau of Meteorology 2015).

4.3.2 Watering quantity

The greywaters and water in control treatments were generally added as needed, often on a daily basis in hot weather. The standard watering volume of 300 ml was approximately the same volume of water which would be deposited to each pot by a little less than 5 mm rainfall. The samples which were given Miracle-Gro® treatment had this applied at a minimum of 14 day intervals, which could be increased by a day or two if sufficient rain fell when Miracle-Gro® application was due. The procedure was to add 200 ml of the Miracle-Gro® solution, followed by 100 ml of tap water a few minutes later, with 300 ml of only tap water being added on watering days in between the 14 day intervals. It did not take long to realise that 5 mm of water added by rain and 5 mm coming from watering on a hot day, did not equate. Samples watered by rain tended to remain moist for longer periods because of factors such as air temperature and humidity, evaporation rate, and rate of addition of water. On a few very hot days the watering quantity, mainly for the Tall Fescue, was sometimes increased to 600 ml. This was done if it was noticed that some samples were showing stress, or if watering would not be possible on the following hot day. The overall aim of watering was to keep the plants as free from water stress as possible. See Appendix B for the monthly volumes of greywaters applied per pot over the experimental period 9/11/07 to 15/3/09.

4.3.3 Watering treatments used on Spring and Summer planted turf

For an explanation of why some different treatments were applied to the Spring and Summer planted turf specimens refer to Section 4.2.5. The treatments that were applied to the turf specimens are indicated by the √ mark in Table 4.1.

Table 4.1 – Water treatments used on Spring and Summer planted turf

Water Code	Spring	Summer	Water Code	Spring	Summer
Water	√		CPTW Shower		√
CPTW	√	√	M/Gro	√	√
CPTWU	√		CPDR	√	
ECTW	√		ECDR	√	
Shower	√	√	SHU	√	√
SHU/5	√		CPTW SHU		√

4.3.4 Reference fertiliser

Miracle-Gro® All Purpose Plant Food was selected as the reference fertiliser because:

1. It was considered to be easier to evenly apply a water soluble fertiliser to turf in a pot than to apply a solid fertiliser.
2. Suitability for its use on lawns and gardens was judged from listed details and instructions printed on an older packet of the fertiliser.

The newer packets bought for the project however did not have as specific application details and just stated “For All Flowers, Vegetables, Trees, Shrubs, Houseplants.”

The plant food had an NPK ratio of 15:13.1:12.4 and the major ingredients listed on the packets were:

1. Mono-Ammonium and Di-Ammonium Phosphates.
2. Urea.

3. Potassium Chloride.

The Miracle-Gro® All Purpose plant food was applied to the turf by first dissolving one level 15 ml scoop of the fertiliser in 4 litres of tap water, and then sprinkling 200 ml of this solution to each pot undergoing Miracle-Gro® treatment. The 200 ml of solution was added from a sprinkling cup while the cup was being moved around over the pot (Refer to Section 4.3 for details). After at least 30 minutes, 100 ml of tap water was added to each Miracle-Gro® pot to bring the added watering volume up to 300 ml. The standard watering volume in normal conditions was 300 ml, and so this procedure ensured that the total volume of water added to the Miracle-Gro® pots was the same as that added to the greywater pots.

The suggested interval for adding Miracle-Gro® is every 7 to 14 days however it was decided to be cautious, especially in hot weather, and to aim for a minimum of 14 days between treatments. Normal tap water was applied on all watering days in between Miracle-Gro® treatments. On a few occasions the addition of Miracle-Gro® and the greywaters was also delayed by a few days because of rain.

4.4 Greywater production

From the outset the aim was to use naturally produced greywaters sourced from a bathroom shower or a laundry for this project, rather than blending up consistent ‘greywaters’ from raw ingredients. The rationale for this was that the aim of the experiments was to produce data that was directly applicable to real life watering with ‘typical’ greywaters, with the associated variables, rather than using ideally formulated solutions. Limited formulation was however necessary when producing equal volume blends of laundry and shower water, and when producing some greywaters with added human urine. No fabric softeners or bleaching compounds were used in the washing of clothes, and contamination of the shower and laundry greywaters by surface cleaning compounds was avoided. The household water consumption increased by about 25% because of the need to regularly produce fresh greywaters. Overall the study was entirely based on using the greywaters and urine produced by the author. As the main purpose of the study was to investigate the feasibility of using greywater on domestic plants, rather than to investigate the range of greywaters that might be produced in domestic situations, and their effects, the decision was made to use greywater from a single source of shower and washing machine. The variation of greywaters and their effects on plant growth, constituted potential follow-up studies, once the feasibility of using greywater of these types had been established.

4.4.1 Laundry sourced greywaters

The laundry greywaters were produced using a Fisher & Paykel MW512 top loading washing machine with a wash load capacity of 5.5 kg, and with three wash water levels. The heavy duty washing cycle was used which consisted of wash, spray rinses, deep rinse, and spin dry. The laundry trough into which the greywaters were pumped had a capacity of 66 litres so usually the low water wash was used for capturing the Total Wash water (wash water plus all the rinse waters), and the medium water level wash was used when capturing only the Deep Rinse water. The captured greywaters were then transferred by bucket to clean plastic 60 litre garbage bins.

Two types of laundry washing compounds were compared in the experiments by making Total Wash and Deep Rinse water from each. The two compounds being:

1. Cold Power® Advanced concentrate laundry powder, which contained phosphate.
2. Earth Choice® laundry liquid, which was phosphate free.

It was accepted that there would normally be some variation between batches of similar types of laundry produced greywaters, so no attempt was made to launder the same type of articles with each wash. Whatever needed washing at the time such as clothes, underwear, and towels was washed just as would be expected in a normal situation. Further variation could result from how much of a laundry compound was added to a wash. A supplied scoop of approximately 110 ml was used to estimate the required amount of Cold Power® powder, and a small marked beaker was used for the Earth Choice® liquid. There was no attempt to dose the water more accurately because it was considered that:

1. Most people doing the washing would use the same or similar method.

2. The dosage recommendations do not differentiate between large and smaller top loading washing machines.

4.4.2 Shower water

The shower greywater was collected in the shower base by blocking the drain outlet with a flexible disc cut from a piece of linoleum. The water was later efficiently scooped into a bucket using a plastic measuring jug and then transferred outside to a clean black 60 litre garbage bin. Collection of the shower water in the shower base ensured that the time taken for a shower was limited, otherwise an overflow could occur. The aim was to evaluate shower water against shower water containing human urine, and not to evaluate different shower greywaters such as those resulting from using either liquid body wash products or solid soaps. Liquid body wash was chosen for this project because of personal preference, and because it generally produced less turbid greywater. Over the study period two types of body wash and one shampoo were used which provided an acceptable variation, to simulate what may occur in a typical household in real life. The body wash and shampoo were squeezed out on to a sponge or hand and not measured out more accurately, so some further acceptable variation could occur between washes. The products used were:

1. Palmolive® Aroma Therapy Shower Gel Sensual.
2. Palmolive® Pure Cashmere Shower Cream.
3. Pears® Balance Care Shampoo for Normal Hair.

4.4.3 Addition of urine to shower water

The shower water and urine blends were never kept in storage. Fresh urine was collected each watering day and added accurately to the shower water with the aid of 1 litre and 50 ml or 100 ml laboratory measuring cylinders. Urine levels of 1% v/v and 0.2% v/v were used in shower waters for the watering of turf, whereas the native flowers were subjected to urine levels of 0.5% v/v and 0.2% v/v.

4.4.4 Greywater blends

The greywater blends were also made up immediately before use and not stored. In general it would be expected that blends of laundry and shower waters would more likely occur in domestic greywater use rather than in community centres. Accepting that in a typical household the ratios of blended laundry and shower sourced greywaters could vary considerably, it was decided to just make simple equal volume blends. The two blends and the plants they were applied to were:

1. Shower water plus Cold Power® Total Wash water – (Turf and Native Flowers).
2. Shower water containing 1% v/v urine plus Cold Power® Total Wash water – (Turf only). This blend therefore contained 0.5% v/v urine.

4.4.5 Storage of greywaters

Five greywater types were stored in clean plastic rubbish bins on the south side of the house that provided protection from the sun. Four of the greywaters were sourced from the laundry i.e. the total wash and deep rinse waters made from the Cold Power® and Earth Choice® compounds, and the fifth greywater was from the shower. The waters could be stored for up to 48 hours with no noticeable odour but were generally replaced frequently, even if not used such as during rain periods. In warmer months an extra shower sometimes had to be taken to provide sufficient supply.

4.4.6 Urine addition to other greywaters

Towards the latter stage of the experiment the turf samples being treated by greywaters without added nutrients (e.g. CPTW, ECTW, Shower, Water, CPDR, and ECDR) were displaying poor growth. CPTW and Shower treated samples were duplicated among the Spring and Summer sets of turf, so a decision was made to select CPTW treated samples sourced from the Spring set, to determine whether the addition of urine at this late stage would encourage better growth. It had been observed as early as the first stage of growing Tall Fescue (9/11/07 to 31/12/07), that the addition of 1% v/v of urine to shower water took about a month for growth and colour improvements in the turf to become noticeable. Therefore initially 1% v/v urine was added to the CPTW Spring set of turf samples to kick start the process, and the level was later reduced to 0.5% v/v urine. The treatment was labelled CPTWU (Cold Power® Total Wash plus Urine).

At the conclusion of the Spring set of experiments, and encouraged by the good growth shown by CPTWU treated samples, a short 14 week experiment (6/12/08 to

12/3/09) was conducted on the poor growing ECTW, Shower, Water, CPDR, and ECDR treated turf samples from the Spring set. Urine at 0.5% v/v level was added to the treatments every time the samples were watered. See Appendix C for the full range of treatments that were used on turf and native flowers in this study.

4.4.7 Increased watering of SHU treated Tall Fescue (Spring) and Kikuyu (Spring)

During the previous summer it was observed that some Tall Fescue turf samples treated with SHU (1% v/v urine in shower water) could suffer if watering with the standard 300ml was missed out or delayed in very hot weather. The problem was first noticed in samples positioned along the outside of a group i.e. those which would be the first to be hit by the hot drying North winds. The problem was also noticed in a couple of samples treated with M/Gro. It was obvious that samples receiving these treatments had far greater growth than the samples receiving the other treatments, and hence they used up more water for growth and through transpiration. The problem with Tall Fescue was controlled by occasionally doubling up the watering level of all Tall Fescue samples to 600 ml i.e. whenever very hot weather was expected or if watering could not be done the next day. The Tall Fescue samples receiving the other treatments did not show problems but still received 600 ml to keep the watering level applied to all Tall Fescue samples constant. Watering delays on expected hot days when samples were cut were avoided by watering the samples before any cutting was done. Kikuyu receiving SHU treatment did not show the same severe problem during very hot conditions. However it was observed that SHU treated Kikuyu could display some signs of wilting about an hour before Kikuyu receiving the other treatments.

Whenever very hot weather was expected it had become a question as to whether 600ml of watering should be applied to the Tall Fescue samples. It was therefore decided to determine whether Tall Fescue samples which had previously been subjected to SHU for more than a year, would require less vigilance during hot weather if 600ml of SHU was applied at every watering session. Because 6 ml of urine would be added to each sample with every 600 ml of SHU, there was some concern that too much urine would be added to the samples during hot spells, causing the samples to suffer. The same experiment was also carried out on Kikuyu samples which had previously been subjected to SHU treatment for 11 months.

4.5 Layout of pots for turf growing experiments

4.5.1 Spring set of turf samples

The eighty pots containing the Spring set of turf samples were laid out into five replicate sets labelled A, B, C, D, E, each of which contained eight pots of Kikuyu and eight pots of Tall Fescue (See Table 4.2 and Figure 4.1). The plan was to apply eight watering treatments i.e. M/Gro, Shower, Water, ECTW, ECDR, CPTW, SHU, and CPDR to these turf samples. The pots of turf for each replicate set were selected by randomly drawing out written numbers from a container.

The pattern of the layout of pots receiving the range of treatments was randomly drawn for only one of the replicate sets (A), because it was considered that if the watering patterns for all the replicate sets were randomly drawn, there was a significantly increased potential for errors in occasionally watering with the wrong treatment across a fully randomised set of trials (there were 150 pots of turf and also later 100 pots of native flowers to be watered, sometimes daily in very drying conditions). The other four replicate sets were therefore watered in patterns that maximised randomisation but minimised the chance of treatment error. This variation in pattern of watering was that replicate sets B and E had their pattern of treatments interchanged (flipped over) along the centre line with the result, for example, that M/Gro on Kikuyu in replicate A was an outside pot whereas M/Gro on Kikuyu in replicate B was an inside pot. This interchange between outside and inside positions was intended to account for any possible differences in drying conditions between inside and outside pots, and to maximise randomisation.

4.5.2 Summer set of turf samples

The sixty pots containing the Summer set of turf samples were arranged into five replicate sets labelled F, G, H, J, K, and each replicate set contained six pots of Kikuyu and six pots of Tall Fescue (See Table 4.2 and Figure 4.1) The treatments applied to the Summer set were SHU, CPTW, Shower, CPTW Shower, CPTW SHU, and M/Gro. The pots of turf samples and the watering patterns were selected in the same way as for the Spring set.

4.5.3 Spring set SHU/5 samples

Excess turf from the Spring set was kept growing on top of sandy loam top soil in small garden beds made from cut out car tyres. On observing the strong growth displayed by turf treated with SHU (1% v/v urine), it was decided to include turf treated with shower water containing only one fifth of the urine level contained in SHU. The new treatment was referred to as SHU/5, and the specimens were interspersed mainly among the Summer planted pots of turf, because there was no room to include them with the Spring set, without expending a considerable amount of work to move and level the pots again.

The photograph (Figure 4.1) and Table 4.2 illustrate the positions of the Kikuyu and Tall Fescue pots, the treatments, the pot and set labels, and how the pots containing the turf samples were positioned in two quadruple rows to the west (right) side of the pots containing the native flowers.

Figure 4.1 – Layout of pots for turf and native flower experiments

Table 4.2 - Layout of pots for Kikuyu & Tall Fescue experiments

	KI	**TF**	**KI**	**TF**						
	K-Set									
K1	SHU	SHU	CPTW	CPTW	K4					
K5	Shower	Shower	CPT Sh	CPT Sh	K8					
K9	M/Gro	M/Gro	CPT SHU	CPT SHU	K12					
	A-Set									
A1	M/Gro	M/Gro	Shower	Shower	A4	**KI**	**TF**	**KI**	**TF**	
A5	Water	Water	ECTW	ECTW	A8	**D-Set**				
A9	ECDR	ECDR	CPTW	CPTW	A12		D17	SHU/5	SHU/5	D18
A13	SHU	SHU	CPDR	CPDR	A16 D1	M/Gro	M/Gro	Shower	Shower	D4
					D5	Water	Water	ECTW	ECTW	D8
	B-Set				D9	ECDR	ECDR	CPTW	CPTW	D12
B1	Shower	Shower	M/Gro	M/Gro	B4 D13	SHU	SHU	CPDR	CPDR	D16
B5	ECTW	ECTW	Water	Water	B8					
B9	CPTW	CPTW	ECDR	ECDR	B12	**E-Set**				
B13	CPDR	CPDR	SHU	SHU	B16 E1	Shower	Shower	M/Gro	M/Gro	E4
					E5	ECTW	ECTW	Water	Water	E8
	C-Set				E9	CPTW	CPTW	ECDR	ECDR	E12
C1	M/Gro	M/Gro	Shower	Shower	C4 E13	CPDR	CPDR	SHU	SHU	E16
C5	Water	Water	ECTW	ECTW	C8					
C9	ECDR	ECDR	CPTW	CPTW	C12	**F-Set**				
C13	SHU	SHU	CPDR	CPDR	C16 F1	SHU	SHU	CPTW	CPTW	F4
					F5	Shower	Shower	CPT Sh	CPT Sh	F8
	G-Set				F9	M/Gro	M/Gro	CPT SHU	CPT SHU	F12
G1	CPTW	CPTW	SHU	SHU	G4		F13	SHU/5	SHU/5	F14
G5	CPT Sh	CPT Sh	Shower	Shower	G8	**KI**	**TF**	**KI**	**TF**	
G9	CPT SHU	CPT SHU	M/Gro	M/Gro	G12					
G13	SHU/5	SHU/5	G14							
	H-Set									
H1	SHU	SHU	CPTW	CPTW	H4		**S**			
H5	Shower	Shower	CPT Sh	CPT Sh	H8		**E W**			
H9	M/Gro	M/Gro	CPT SHU	CPT SHU	H12		**N**			
		H13	SHU/5	SHU/5	H14					
	J-Set									
J1	CPTW	CPTW	SHU	SHU	J4					
J5	CPT Sh	CPT Sh	Shower	Shower	J8		**CODES:**			
J9	CPT SHU	CPT SHU	M/Gro	M/Gro	J12		**KI** = Kikuyu turf			
J13	SHU/5	SHU/5	J14				**TF** = Tall Fescue turf			
	KI	**TF**	**KI**	**TF**			CPT Sh = CPTW Shower			
							CPT SHU = CPTW SHU			
	Scaly Buttons and Small Vanilla									
	Lilies (8 Pots)									

4.6 Tools for cutting and collection of turf samples

4.6.1 Frame for standardising the cutting

An aluminium frame was constructed from a 3 mm x 40 mm flat bar for use as a guide to cutting equal areas of turf from within the pots. The frame was 40 mm high and covered an area of approximately 180 mm x 180 mm and so ensured that turf samples could be cut away from the edges of the 250 mm x 250 mm pots. All around the outside of the frame a piece of woven plastic cloth was attached so that grass outside the frame would be covered, and not accidentally cut and sucked up by the vacuum collection system used. The grass was cut so that it was level with the top of the frame, using flat blade scissors. Turf cut heights with the use of the frame tended to average about 45 mm for Tall Fescue and 50 mm for Kikuyu. A photograph of the frame used is shown in Figure 4.2 below (Note: this shows the frame sitting on the ground between the pots. The grass is not a cut sample).

Figure 4.2 – Frame that was used as a guide for cutting turf

The frame was not of a perfect square shape but was calculated to enclose an area of 331cm^2 by carefully tracing the frame shape on several pieces of 80 gsm paper, and then comparing the weights of the paper cut outs against the weights of full sheets.

The area was also confirmed by taking several measurements to estimate the average dimensions of the frame. To calculate the growth rates per m^2 a factor of 30.2 was therefore determined from the $331cm^2$ area enclosed by the frame.

4.6.2 Vacuum collection system for cut turf

A suction system was used to collect the grass as it was being clipped. The system was constructed by attaching the outlet of an external “Turbo Dust Filter” to the suction hose of a vacuum cleaner and then connecting a flexible piece of swimming pool filter hose to the inlet of the Turbo Dust Filter. The grass clippings did not reach the vacuum cleaner but were caught in the collection chamber of the Turbo Dust Filter, from which they were easily tipped into a paper bag. About five clippings always got caught in the hose where it was attached to the inlet of Turbo Dust Filter. This was not a problem because the hose was easily removable for collecting the clippings. The unbranded Turbo Dust Filter was purchased from a local Godfreys vacuum cleaner store. The vacuum collection system is shown in Figure 4.3 below.

Figure 4.3 – Vacuum collection system for cut turf

The presence of dew often delayed the cutting of the grass until mid to late morning because pieces of wet grass tended to stick inside the hose and to the walls of the collection chamber. In the early stages of the experiments, to avoid further wetting of the grass, the watering was suspended until after the cutting, but this method almost resulted in the loss of a few pots of grass on a hot day. The procedure of watering first thing in the morning on grass cutting days was therefore adopted, and by the time all watering was completed the first watered pots were usually dry enough for cutting.

4.6.3 Extra tools used

The following extra tools were needed to cut and collect the turf:

1. **Small brush attachment from the vacuum cleaner –** The turf samples were gently vacuumed before cutting to remove contaminants that could affect the weight of collected turf, and to free up some tangled blades of grass. Common contaminates included loose sand particles, small leaves, occasional insect, and some dry turf clippings that may have resulted from the previous trimming of the turf sections along the edges of the pots. The brush attachment was used because it provided a gentler contact surface than the end of the hose, and so minimised damage to the uncut grass undergoing suction.
2. **Solid house brick** – The brick was placed on the frame as a means of keeping the frame in place and applying some pressure. The brick also served as a rest for the hand holding the vacuum hose just above the grass, and also provided a cutting guide away from the edges of the frame. After cutting the exposed grass within the frame, the brick was repositioned to expose other sections. Initially U-shaped wire clips were used to keep the frame from moving however the brick method was considered to be superior.
3. **Flat blade scissors** – These commonly available scissors cut close to the frame and were cheap enough to be frequently replaced when needed.
4. **Plastic watering spike** – This was a round and tapered spike normally intended as an attachment to a plastic bottle for sub surface watering of plants. It was very useful to reposition some turf that obviously may have been pushed to the wrong side of the frame when placing it down.

4.7 Cutting the turf and determining the dry weight and growth height

Because the turf was predicted to grow rapidly for at least some treatments, an integral part of the experimental design was to cut the turf and collect the clippings in a standard way, as would happen with any well-tended turf or lawn. To quantify growth two methods used were; (i) determining the dry weight of cut turf, and (ii) measuring the height of turf growth between cutting sessions.

There were a total of 150 pots containing turf from the Spring and Summer Planting sets, and because the average time taken from step 1 to step 8 below was 15 minutes for each pot, the cutting had to be done over several days. Wet grass or rain delayed or postponed the cutting, and often up to 3 hours of watering the turf and 100 pots of native plants had to be fitted in with the cutting. The procedure for cutting the turf and obtaining dry weight and growth height was:

1. Connect brush attachment to the hose leading to the inlet of the Turbo Dust Filter and gently vacuum away any stray dead pieces of turf or other contaminants in the pot. Empty the Turbo Dust Filter and remove the brush.
2. Take several height measurements of the turf using the marked stick as a guide.
3. Place frame centrally on the turf in the pot and free up any grass that appears to have been pushed to the wrong side of the edges of the frame.
4. Place a brick across the centre of the frame pointing away from one self, rest hand on the brick and hold the vacuum hose close to the grass. Start the vacuum cleaner and begin cutting the grass with the scissors using the top edges of the frame, and the bottom of the brick as a guide for cutting height.
5. Switch hands for holding the scissors and the hose and cut on the other side of the brick. Reposition brick until all the grass inside the frame is cut.
6. Switch vacuum cleaner off, unclip the collection chamber of the Turbo Dust Filter and empty the grass into a labelled brown paper lunch bag. Carefully remove the inlet hose from the filter and shake the few trapped clippings into the paper bag.

7. Close the bag with about three folds and place in a bucket, and then measure the height of the cut turf from the ground surface.
8. Remove the frame and trim the uncut sections of the turf along the edges of the pot to be level with the cut area. Remove and dispose of the excess cut turf.
9. Hang the bags of cut turf by one corner on a string line in a sheltered position such as a shed, veranda, or room. Leave to air dry for at least a couple of weeks or longer if possible especially in colder weather.
10. Place bags with air dried turf in a laboratory oven at 70°C for 24 hours.
11. Weigh the oven dried turf together with the bag, empty the turf on to a large piece of paper and ensure that any remaining pieces of turf are shaken out.
12. Weigh the empty paper bag and calculate the weight of dry turf by subtraction.

The following photographs (Figure 4.4 to Figure 4.9) illustrate how the turf samples were cut. The scissors are shown without being held by a hand because the hand was used to hold the camera.

Figure 4.4 – Uncut turf sample (Kikuyu with SHU treatment)

Figure 4.5 – Use of Spike for freeing up turf around frame

Figure 4.6 – Vacuum method of collecting turf as it is being cut

Figure 4.7 – Turbo Dust Filter full of cut turf from within frame

Figure 4.8 – Turf appearance after removal of frame

Figure 4.9 – Turf appearance after trimming the edges

4.8 Determining growth of turf

Determining the dry weight of cut turf was the main method relied upon for obtaining turf growth results because it took into account the density of the growth as well as the variation in heights within a sample. This method however took a considerable amount of time to accurately cut, collect, dry, and weigh the samples, so the cutting of turf was mainly carried out when the well growing specimens produced considerable growth. From the dry weight of cut turf the average daily growth rate in g/m^2 was determined for each growth period, and also a running total dry weight of collected turf was able to be plotted. Measuring the growth of grass by determining the dry weight is common across a wide range of studies – for example see (Alfiya et al. 2012; Lunt & Morgan 1999; Wherley 2011).

4.8.1 Daily growth rates

On each occasion the cutting of a complete set of turf samples generally could not be done on the one day and so if some samples grew for a few days longer the cut weights of those samples could increase. The problem was minimised by calculating the growth rate of turf per day for each sample. In this way if one sample grew 30 days between cuts and another sample 33 days, the dry weights were divided by 30 and 33 respectively to provide the daily growth rates. A frame enclosing an area of 331 cm^2 was used as a guide for cutting the same area of turf each time. The enclosed area fitted into a square metre 30.2 times and so the results were multiplied by 30.2 to determine the daily growth rates as (g/m^2 dry weight). From the daily growth rates the average daily growth rates and the standard deviations were calculated.

4.8.2 Running total growth heights

The secondary method of estimating growth results was by measuring the growth height between turf cutting events. A measuring stick with markings at 1 cm intervals was constructed for this work. The measurements were taken each time the turf samples were cut. In this method the turf height in each pot was measured several times and the 3 highest measurements were averaged. The height of the turf after cutting was also measured and subtracted to give the growth height of the turf in the pot. The growth heights of the five replicates from each treatment were then averaged. Measurement of plant height or stem length to determine plant growth has been commonly used, whether it be for grass pasture or for vegetables (Meat & Livestock Australia Ltd 2013; Misra, Patel & Baxi 2010; Salukazana et al. 2006).

Although quick results were obtained by measuring the turf height, the procedure did not allow for growth densities, and the variation of heights within a sample meant that the elongation of leaves was a broad estimate. Nevertheless, comparisons between the two methods of (i) measuring the growth heights and of (ii) obtaining the dry weights of the turf clippings were possible. The results obtained by measuring the turf growth heights at each cutting session were summed and plotted as running total growth heights.

4.8.3 Running total dry weights

The average results of dry weights of turf clippings from each growth period were summed and presented as running totals from Day 0 to the end of each experiment. The graphs containing the running total dry weights of turf clippings were used as a standard for comparison against the graphs containing the running total growth heights of the turf. The intention was to determine whether the faster method of measuring the growth heights of turf would give acceptable results if it was not feasible to determine the dry weights of turf clippings.

4.9 Pests on turf specimens

4.9.1 Weeds

Some broad leaf weeds and a thin intertwining and spreading type grew in a few of the Kikuyu pots. The broad leaf weeds were easily seen and removed by hand, but the thin spreading type was harder to notice and had to be traced back to where it was rooted to the soil to be effectively pulled out. This weed tended to regrow probably because all traces were difficult to remove. It was not known whether the Kikuyu samples were affected after planting into pots or whether the supplied Kikuyu contained the seeds. All unwanted plant infestation in the pots was minor for the duration of the experiments.

4.9.2 Birds

A brown bird was often seen on the pots of turf mainly around nesting time in Spring 2008. Small moths and tiny snails, plus the odd worm could sometimes be found among the turf. The problem that the bird caused was to sometimes leave a bird dropping behind on top of the turf, usually near the edges of the pots. Whenever a bird dropping was noticed it was carefully removed, however it is uncertain whether all droppings were found. It may explain why a few pots with poor growing turf had small sections with vigorous growth especially near the edges.

The following Chapter 5 presents the growth results produced by several specific types of greywaters on Tall Fescue turf. Extra experimental work in using urine to regenerate growth, and statistical analysis of results are also presented.

Chapter 5: Results of using Greywaters on Tall Fescue Turf

5.1 Introduction

The experiments determining the effects of greywaters on turf growth were carried out on Tall Fescue and Kikuyu turfs planted in both Spring and Summer 2007. The results obtained with Kikuyu turf are reported separately in Chapter 6. The results are presented in graphical form as Figures to make it easier to quickly see differences in growth rates and patterns of growth. However, if required the corresponding numerical data for Tall Fescue in Table form can be obtained from the author.

In these experiments the turf samples were watered with the treatments outlined in Chapter 3 (Section 3.5) and in Table 4.1, with addition of other water via the occasional rain event. Two of the treatments, Miracle-Gro® All Purpose plant food solution and Water, were not greywaters but provided nutrient and non-nutrient based references for comparison against the greywaters used. Each treatment was applied to five replicates which were dispersed among pot groups A, B, C, D, E, for the Spring planted samples, and F, G, H, J, K, for the Summer planted samples. For the late inclusion of SHU/5 treatment (0.2% v/v urine in shower water), the Spring planted turf samples were located among groups F, G, H, D, J. The main method of assessing the growth was to collect the turf clippings each time the turf was cut, and then to determine their dry weights. From these figures the daily growth rates, and the running total dry weights of clippings could be calculated. The secondary method of determining growth was to measure the growth heights between cutting sessions, from which the running total growth heights could be calculated.

The growth rates are presented in the Figures below and cover several growth periods during each experiment. The presented growth periods are mainly between two consecutive cutting sessions; however some growth periods were combinations of more than one cutting session, especially if some treatments grew a lot faster than others. The Figures show the average daily growth rates, with error bars depicting the standard deviations.

It should be noted that for each experimental set the Figures are presented with the same vertical scaling, rather than using optimum scaling for each growth period. This is done deliberately in order to provide a quick visual comparison of how the growth rates changed between growth periods, and between treatments.

The average daily growth rates are expressed in the Figures with slightly overlapping dates i.e. in Figure 5.2 the stated growth period is (30/12/07 to 19/4/08) and in Figure 5.3 the period is (17/4/08 to 10/7/08). The dates throughout this thesis are expressed in the format generally used in Australia i.e. Day/Month/Year. The first date in each Figure indicates when the *first sample* was cut at the *start* of the respective growth period and the second date indicates when the *last sample* was cut at the *end* of that growth period. Therefore from Figure 5.2 and Figure 5.3 it can be deduced that in April 2008, the cutting of the Tall Fescue (Spring) samples occurred over the three days 17/4/08 to 19/4/08.

5.1.1 Analysis of results

The analysis of Tall Fescue growth results presented in this Chapter 5, and of Kikuyu results presented in Chapter 6 were performed by the same methods. The Average or Mean growth results, and the Standard Deviations were calculated by using Microsoft Office Excel 2003. One-way ANOVA tests were done with an online calculator (CSBSJU) available from *The College of Saint Benedict and Saint John's University*, and occasionally Student's t-Tests were completed with another online calculator (CSBSJU) also available from the same source. The two CSBSJU calculators expressed the result of each statistical calculation as a probability, assuming the null hypothesis, that there was no difference produced by the treatments. A probability (p) greater than 0.05 signified that there was no statistically significant difference produced by the treatments, while a probability less than 0.05 was taken as showing that there was a statistically significant difference between treatments.

5.2 Average Daily Growth Rates of Tall Fescue (Spring)

The results presented in Figures 5.1, 5.2, 5.4, 5.5, and 5.7 cover five growth periods from 9/11/07 to 27/11/08. The experiment was ended when the turf samples unevenly sprouted quick growing tall seed stalks. The following discussions are based on the average results shown in the Figures.

5.2.1 Late Spring to Early Summer: Growth period 9/11/07 to 31/12/07

The only significant difference in growth rates produced by the treatments was between those treated with either water or greywater, and those treated with urine containing greywater or with Miracle-Gro® plant food (Figure 5.1). Greywater alone, regardless of type, mostly showed small increases over the water treatment, most of which were not statistically significant (see below), and no greywater treatment showed lower growth rates than water. This pattern was repeated for all the time periods over which the experiment was conducted.

It took about 4 weeks for the effects of 1% v/v urine in shower water to become visibly noticeable in the Tall Fescue as increased growth and deeper green colouration. The growth results over this 7.5 week period are shown in Figure 5.1. The following observations were made:

- Shower produced 29.1% more growth than Water. Combined statistical comparison of average daily growth produced by the six water and wastewater treatments without added urine or plant food i.e. Water, Shower, ECTW, ECDR, CPTW, and CPDR showed no significant difference ($p = 0.192$).
- Total wash ECTW produced 12.9% more growth than deep rinse ECDR, whereas the opposite occurred with CPDR showing a 14.5% increase over CPTW, although in neither case were the results statistically significant ($p = 0.364$ and $p = 0.290$ respectively).
- The urine containing treatment SHU produced an average daily growth of approximately twice that of the treatment Shower, and also of the other five greywater treatments. Result was statistically significant ($p < 0.001$).
- SHU produced 34.4% more growth than the next highest M/Gro ($p = 0.004$).

- The effect of plant food was shown by M/Gro producing 89.6% more growth than Water, which gave the lowest growth ($p = 0.001$).
- Non-phosphate based total ECTW produced 23.1% more growth than phosphate based CPTW ($p = 0.025$).

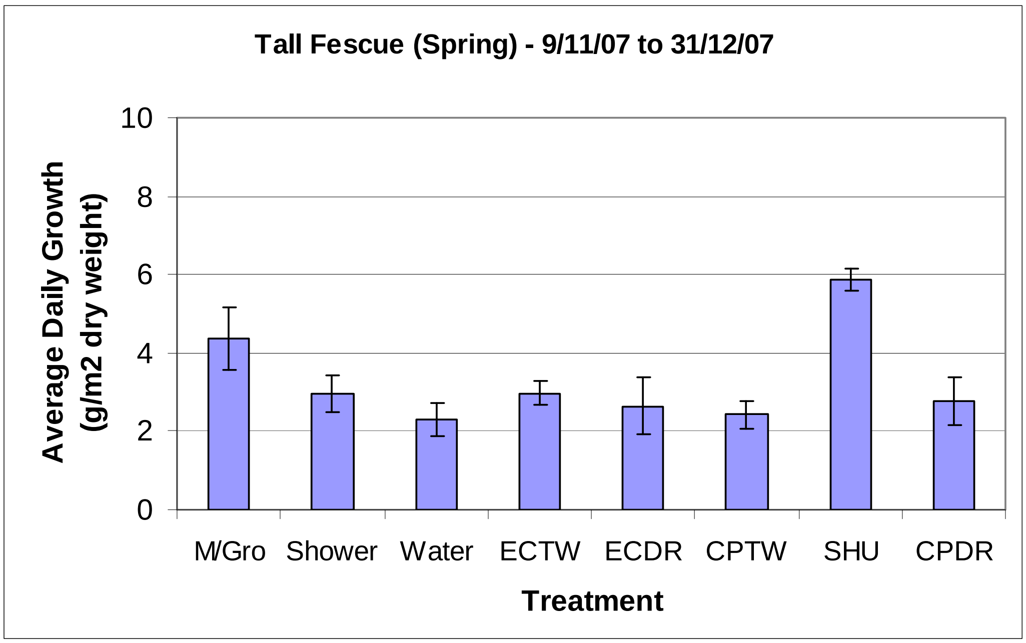

Figure 5.1 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Spring) over period 9/11/07 to 31/12/07 (Error bars indicate one standard deviation)

5.2.2 Summer to Mid Autumn: Growth period 30/12/07 to 19/4/08

The overall pattern of results in this section is that only greywaters with added urine or the Miracle-Gro® plant food treatment, showed order of magnitude increases in growth rate results (Figure 5.2). All greywaters without added urine produced some increases in growth rates over water alone – these are reported in more detail below. The 15.5 week period included SHU/5 treatment (0.2% v/v urine in shower water) for the first time. The results are shown in Figure 5.2. It was determined that:

- Shower produced 149% more growth than Water, which was statistically significant ($p = 0.006$).
- Water produced the lowest growth.

- Total wash CPTW produced statistically significant more growth than deep rinse CPDR ($p = 0.007$), and although ECTW produced more growth than ECDR the result was not statistically significant ($p = 0.519$).
- The effect of plant food had increased with M/Gro producing 274% more growth than Water ($p < 0.001$).
- The effect of urine on average daily growth was now well pronounced, with SHU and SHU/5 respectively producing 367% and 73.6% more growth than Shower.
- SHU and SHU/5 also respectively produced 210% and 15.3% more growth than M/Gro, with only the difference between SHU/5 and M/Gro results being not statistically significant ($p = 0.086$).
- Phosphate based CPTW produced 30.1% more growth than non-phosphate based ECTW, however result was not statistically significant ($p = 0.068$).

Differences between the average daily growth produced by the six treatments without urine or plant food i.e. Water, Shower, ECTW, ECDR, CPTW, and CPDR, were statistically significant ($p = 0.001$). The deep rinse waters CPDR and ECDR can be considered to be dilute versions of the total wash waters CPTW and ECTW. Splitting the above six treatments into 2 groups however showed that the differences in growth were not statistically significant between the *concentrate* treatments i.e. Shower, ECTW, and CPTW ($p = 0.176$), or between the *dilute* treatments i.e. Water, ECDR, and CPDR ($p = 0.225$).

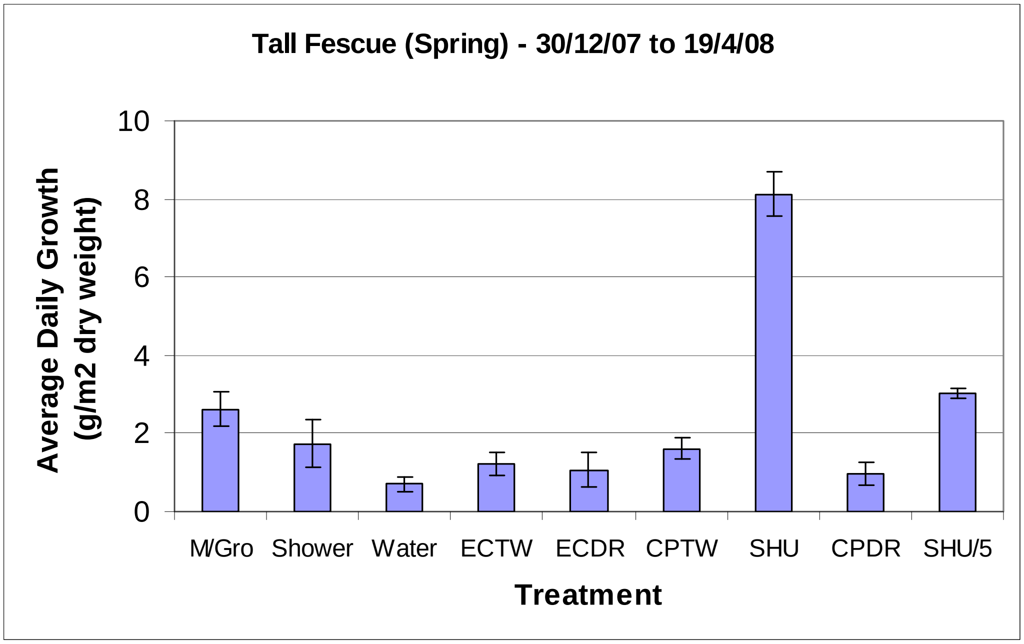

Figure 5.2 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Spring) over period 30/12/07 to 19/4/08 (Error bars indicate one standard deviation)

Differences in growth resulting from the treatments are evident in the following photograph (Figure 5.3) of the B Set of samples in which the Tall Fescue samples are even numbered and the Kikuyu are odd. The Tall Fescue samples had five weeks of early autumn growth since the cutting session on 5/3/08, and the Kikuyu almost six weeks from 29/2/08.

In comparing the SHU treated B15 and B16 respectively with the Shower treated B1 and B2 it can be seen (refer to Figure 5.3) that the addition of 1% v/v urine resulted in considerably more growth in Tall Fescue and also in Kikuyu (results reported in detail in the next chapter), and especially for the Tall Fescue, a much darker green colour development. The two unmarked A Set samples immediately to the right of B1 and B2 were also treated with SHU. The effect of adding plant food can be seen by the M/Gro treated B3 and B4 producing more growth than the Water treated B7 and B8 samples, and especially for the Tall Fescue, development of a darker green colour.

Figure 5.3 – B Set of Tall Fescue (Spring) and Kikuyu (Spring) samples

Table 5.1 – Key to Figure 5.3

Kikuyu **>**	B13 CPDR	B9 CPTW	B5 ECTW	B1 Shower
Tall Fescue **>**	B14 CPDR	B10 CPTW	B6 ECTW	B2 Shower
Kikuyu **>**	B15 SHU	B11 ECDR	B7 Water	B3 M/Gro
Tall Fescue **>**	B16 SHU	B12 ECDR	B8 Water	B4 M/Gro

5.2.3 Mid Autumn to Mid Winter: Growth period 17/4/08 to 10/7/08

Over this 12 week period all the treatments produced lower growth rates than those recorded for summer to mid-autumn. Figure 5.4 shows that:

- Shower produced 283% more growth than Water, which was statistically significant ($p = 0.034$).
- Water again produced the lowest growth.
- Total washes CPTW and ECTW produced very small growths, although these were greater than produced by the deep rinses CPDR and ECDR. The result between CPTW and CPDR was statistically significant ($p = 0.028$), while the result between ECTW and ECDR was not statistically significant ($p = 0.244$).
- M/Gro produced 1617% more growth than Water.
- M/Gro produced 30.4% more growth than SHU/5, which was not a statistically significant difference ($p = 0.124$).
- SHU and SHU/5 respectively produced 1204% and 243% more growth than Shower.
- SHU still produced the largest average daily growth which was 191% more than produced by M/Gro, which had the next largest growth ($p < 0.001$).
- Phosphate based CPTW produced 38.1% more growth than non-phosphate ECTW, which was not a statistically significant growth difference ($p = 0.204$).
- The obvious explanation for this pattern of results is that the turf samples treated with Water or the greywaters without added urine were providing water as well as the water-alone control, but were starting to lack sufficient nutrients to drive plant growth, towards the end of the experiment.

Differences between the average daily growth produced by the five greywaters Shower, ECTW, ECDR, CPTW, and CPDR were not statistically significant ($p = 0.120$), however when the results produced by Water were included, the probability dropped below the threshold to 0.017. This means that all sources of water from wastewater led to growth that was higher than for the water-alone control. As in the previous growth period, the differences were not statistically significant between the *concentrate* treatments Shower, ECTW, and CPTW ($p = 0.516$), or between the *dilute* treatments Water, ECDR, and CPDR ($p = 0.385$).

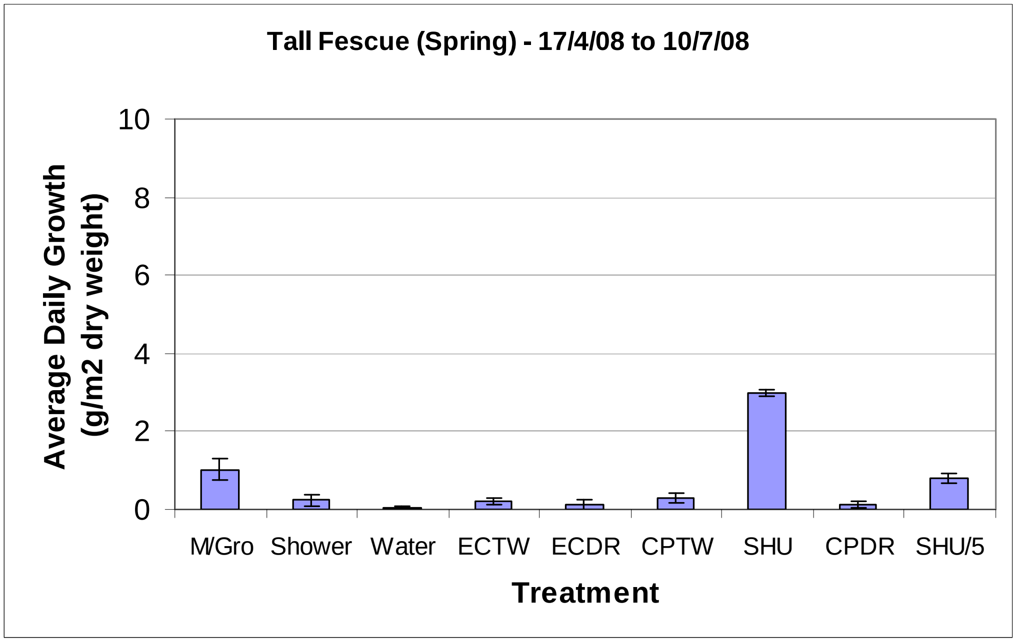

Figure 5.4 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Spring) over period 17/4/08 to 10/7/08 (Error bars indicate one standard deviation)

5.2.4 Mid Winter to Mid Spring: Growth period 9/7/08 to 12/10/08

The results for this 13 week period are shown in Figure 5.5, and it can be seen that:

- Replicates treated with Water or the greywaters without added urine appeared to be limited by nutrients, even though Shower produced 443% more growth than the Water alone control.
- Total wash ECTW still managed to produce 95% more growth than ECDR, which was not a statistically significant difference ($p = 0.423$).
- M/Gro produced 2957% more growth than the Water only treatment, which hardly produced any growth over the six months from 17/4/08.
- SHU still produced the largest average daily growth, which was now reduced to 58.9% more than the next largest M/Gro ($p = 0.001$).
- SHU and SHU/5 respectively produced 795% and 153% more growth than Shower.
- The major changes to the average daily growth, compared to previous period, were shown by CPTWU and M/Gro.

CPTWU treated samples were previously treated by CPTW. However from 31/7/08 urine was added to these samples at a level of 1% v/v to stimulate growth. This was done to see whether treatments with low growth could be stimulated by addition of urine – that is, to test whether these treatments had a deficit of nutrients. Over the 10 weeks from 31/7/08 to 11/10/08 there were 25 watering sessions that added a total of 75 ml urine to each CPTWU pot. From 12/10/08 the urine content of CPTWU was reduced to 0.5% v/v because the samples were now growing satisfactorily and it was felt that 1% v/v urine could cause problems in the coming hot weather.

Statistical analysis showed that the differences between the average daily growth produced by the five treatments Shower, Water, ECTW, ECDR, and CPDR were not statistically significant ($p = 0.207$). Further analysis comparing the three treatments M/Gro, CPTWU, and SHU/5 showed statistically significant average daily growth differences ($p = 0.010$).

The average daily growth of the M/Gro treated samples more than doubled compared to the previous period, whereas that of SHU treated samples only increased by 13%. M/Gro was added on a regular basis with a minimum of 14 day intervals, whereas SHU (containing 1% v/v urine) was added only whenever watering was required. Over the cooler months the need for watering, and hence addition of urine to the turf, was therefore less.

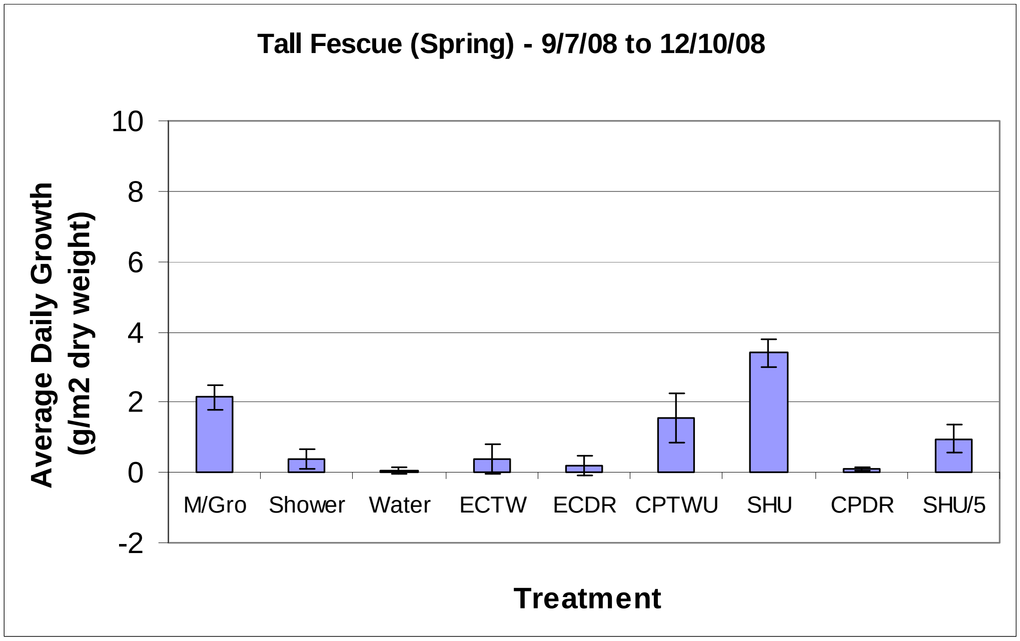

Figure 5.5 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Spring) over period 9/7/08 to 12/10/08 (Error bars indicate one standard deviation)

The following photograph (Figure 5.6) shows the effect of urine on ten weeks of growth from mid winter to early spring (9/7/08 to 18/9/08) of two Tall Fescue (Spring) samples. The SHU treated A14 was showing very good growth and deep green turf colour, whereas the Shower treated B2 was struggling, probably due to a lack of adequate nutrients. Samples receiving the treatments; Water, CPDR, ECDR, and ECTW also showed poor growth and so did not receive a cut at this stage, but were allowed to grow until the next cutting session in mid October.

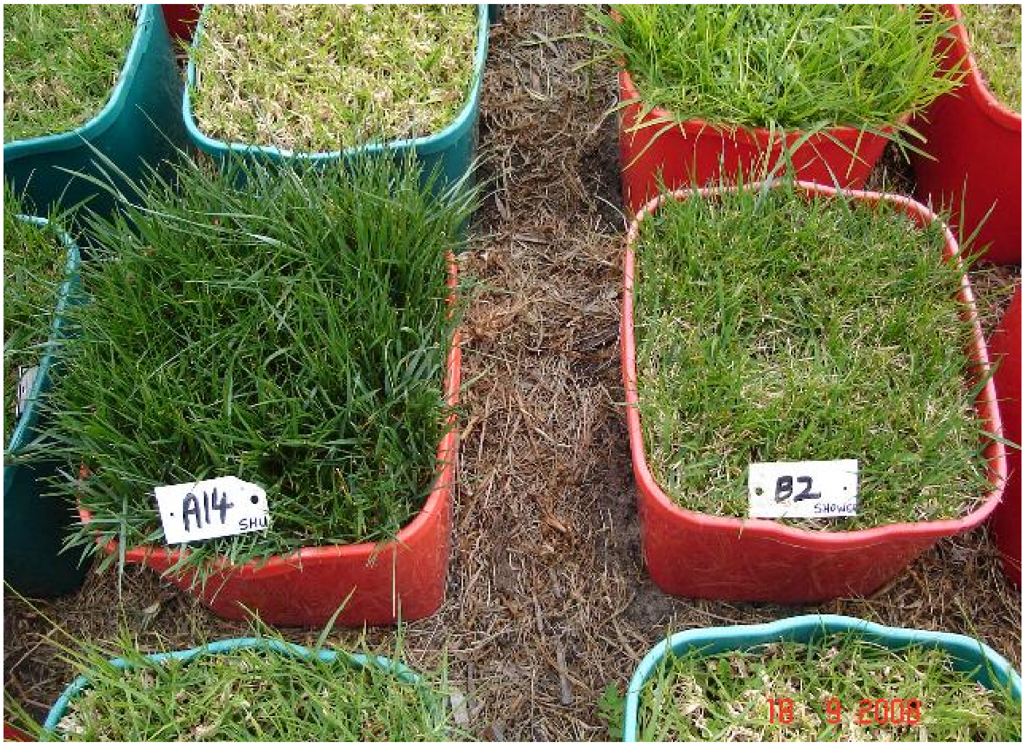

Figure 5.6 – Tall Fescue (Spring) samples showing difference in growth and colour between turf that received urine containing SHU (A14), and non-urine Shower (B2) treatments

5.2.5 Second Year of Spring: Growth period 10/10/08 to 27/11/08

Figure 5.7 covers the growth during the second half of spring 2008. Tall flowering stalks quickly sprang up unevenly throughout the pots (see the photograph Figure 5.8), and so it was decided to end the experiment at this stage. The stalks were removed and the samples showing poor growth were prepared for a new short experiment to determine whether the addition of urine would regenerate growth in these samples.

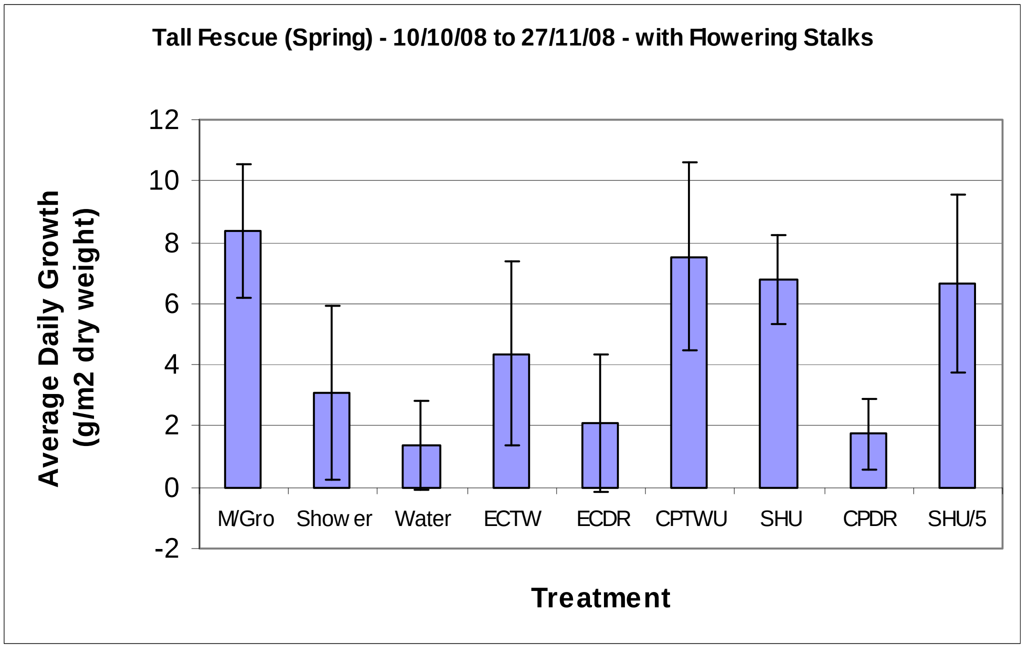

Figure 5.7 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Spring), including Flowering Stalks, over period 10/10/08 to 27/11/08 (Error bars indicate one standard deviation)

Figure 5.8 – Tall Fescue (Spring) samples with Flowering Stalks

5.3 Average Daily Growth Rates of Tall Fescue (Summer)

This experiment investigated the effect on growth of Tall Fescue when a phosphorus containing laundry water (CPTW) and water from the shower (with and without urine) are blended before being used for watering. This would normally occur with watering systems that store greywater before use, but may have some application in simple direct feed greywater systems, which feed out laundry and shower waters separately to the same areas. The two blends of greywaters CPTW SHU and CPTW Shower are compared against the component waters CPTW, SHU, and Shower, and against M/Gro treatment. The blend CPTW SHU therefore theoretically contained half the quantity of added urine that SHU did, and half the laundry based phosphorus of CPTW.

The results presented in Figures 5.9, 5.10, 5.12, 5.14, 5.17, 5.18, and 5.19 cover seven growth periods from 3/1/08 to 28/1/09. The experiment progressed through the flowering stalk stage in November 2008, after which the stalks were removed and the experiment continued on the remaining turf.

5.3.1 Mid Summer: Growth period 3/1/08 to 30/1/08

The results for this initial 4 week period are displayed in Figure 5.9, and show that:

- SHU produced the largest average daily growth followed in order by CPTW SHU, M/Gro, CPTW, Shower, and CPTW Shower.
- The effect of urine was already evident by SHU producing 76.4% more growth than Shower ($p < 0.001$), and CPTW SHU producing 60.3% more growth than CPTW Shower ($p = 0.01$).

Differences in average daily growth produced by the three treatments without added urine or plant food i.e. CPTW, Shower, and CPTW Shower, were not statistically significant ($p = 0.353$). The three treatments containing added urine or plant food i.e. SHU, M/Gro, and CPTW SHU produced growth results that differed by statistically significant amounts ($p = 0.035$). The difference in results between SHU and M/Gro treatments was statistically significant ($p = 0.013$), but not between M/Gro and CPTW SHU ($p = 0.571$).

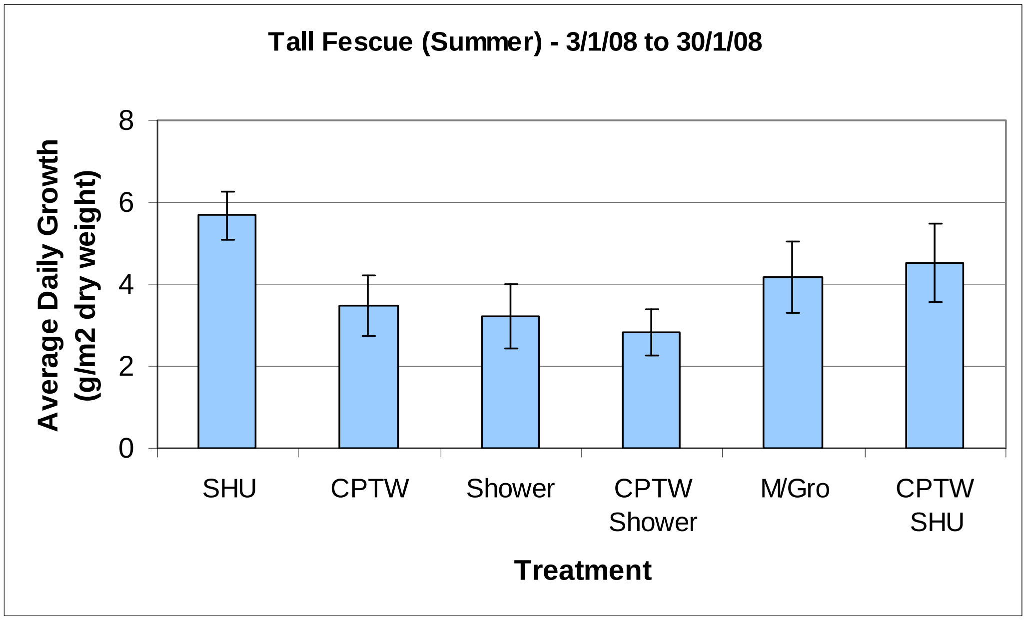

Figure 5.9 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 3/1/08 to 30/1/08 (Error bars indicate one standard deviation)

5.3.2 Late Summer to Early Autumn: Growth period 28/1/08 to 28/3/08

The results for this 2 month period are shown in Figure 5.10. It can be seen that:

- The effect of urine on average daily growth became more pronounced, with SHU producing 249% greater growth than Shower, and CPTW SHU producing 130% more than CPTW Shower.
- SHU was the dominant treatment giving 50.9% greater growth than the next highest CPTW SHU ($p = 0.001$), and 134% more than M/Gro ($p < 0.001$).
- The lowest average daily growth was shown by CPTW Shower, and by Shower.

Differences in average daily growth produced by the three treatments CPTW, Shower, and CPTW Shower were not statistically significant ($p = 0.252$). The three treatments containing added urine or plant food i.e. SHU, M/Gro, and CPTW SHU produced growth results that differed by statistically significant amounts ($p < 0.001$).

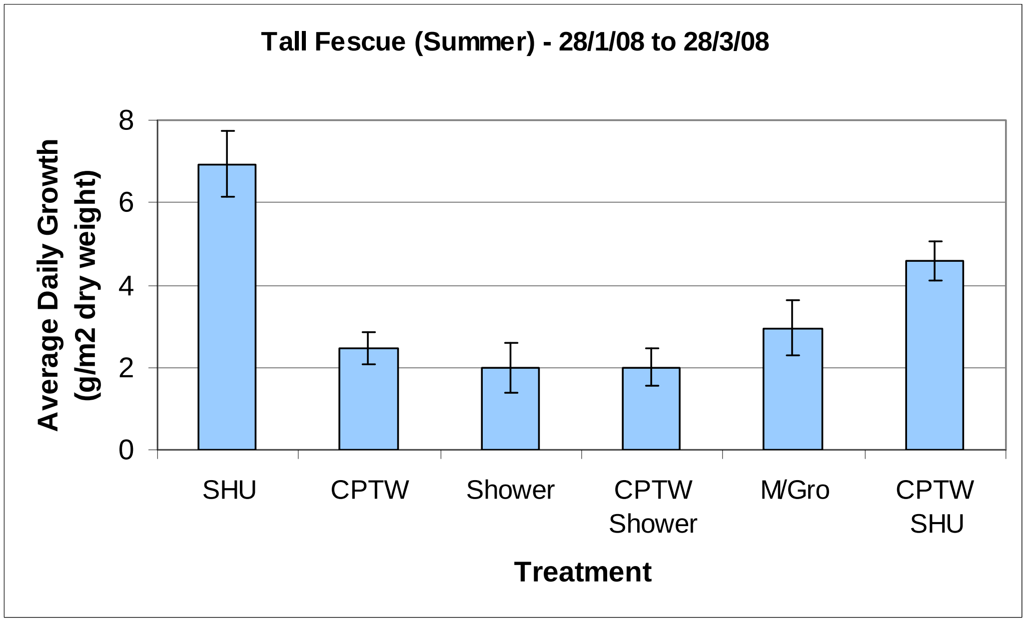

Figure 5.10 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 28/1/08 to 28/3/08 (Error bars indicate one standard deviation)

The differences in growth can be seen in the following photograph (Figure 5.11) of the G Set of samples in which the Tall Fescue samples are even numbered, and the Kikuyu are odd. The Tall Fescue (Summer) samples experienced 30 days of growth since the cutting session on 19/2/08, and the Kikuyu (Summer) samples had grown for 26 days from 23/2/08. Located in the top left side are also the SHU/5 treated samples Kikuyu (Spring) G13, and Tall Fescue (Spring) G14.

The effect of 1% v/v added urine can be seen by G3 and G4 producing more growth and deeper colour than respectively G7 and G8, and the effect of 0.5% v/v urine is demonstrated by G9 and G10 producing more growth and deeper colour than respectively G5 and G6.

Figure 5.11 – G Set of Tall Fescue (Summer) and Kikuyu (Summer) samples

Table 5.2 – Key to Figure 5.11

Kikuyu **>**	G13 SHU/5	G9 CPTW SHU	G5 CPTW Shower	G1 CPTW
Tall Fescue **>**	G14 SHU/5	G10 CPTW SHU	G6 CPTW Shower	G2 CPTW
Kikuyu **>**		G11 M/Gro	G7 Shower	G3 SHU
Tall Fescue **>**		G12 M/Gro	G8 Shower	G4 SHU

5.3.3 Autumn: Growth period 25/3/08 to 19/5/08

The results for this eight week growth period are shown in Figure 5.12. It can be seen that:

- The average daily growth produced by all six treatments decreased compared to the previous period from late summer to early autumn.

- SHU was still the dominant treatment producing 52.8% more growth than CPTW SHU, and 211% more than M/Gro.
- The lowest average daily growth was again due to CPTW Shower and to Shower.
- The effect of urine had increased with SHU now producing 605% more growth than Shower, and CPTW SHU showing 386% more growth than CPTW Shower.

Differences in average daily growth produced by the three treatments CPTW, Shower, and CPTW Shower were not statistically significant ($p = 0.061$). The three treatments containing urine or plant food i.e. SHU, M/Gro, and CPTW SHU again produced growth results that differed by statistically significant amounts ($p < 0.001$).

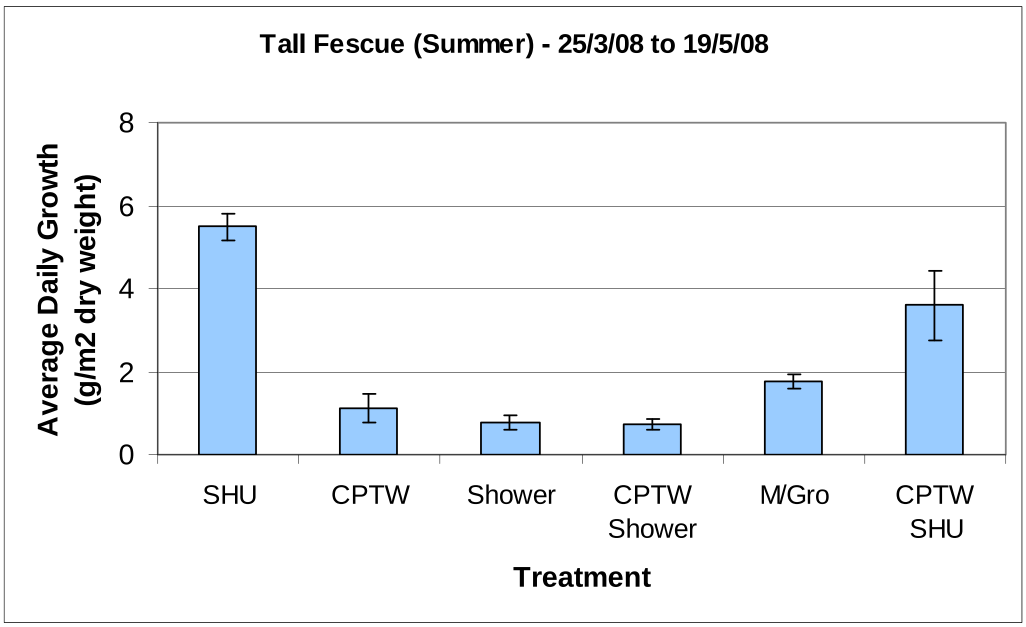

Figure 5.12 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 25/3/08 to 19/5/08 (Error bars indicate one standard deviation)

The following photograph (Figure 5.13) is of two Tall Fescue (Summer) samples from the F Set, which had experienced 7.5 weeks of growth during autumn. It can be seen that the 0.5% v/v urine containing CPTW SHU treatment produced more growth and slightly deeper green turf colour than the urine free CPTW Shower treatment. The only difference between the two treatments was the urine content.

Figure 5.13 – Tall Fescue (Summer) samples showing greater growth and deeper colour produced by CPTW SHU (F12) treatment than by CPTW Shower (F8)

5.3.4 Late Autumn and through whole of Winter: Growth period 18/5/08 to 4/9/08

Over this 15.5 week period it was of no surprise that compared to the previous period of growth during autumn, the average daily growth produced by all six treatments decreased. M/Gro which was regularly added at 14 day intervals produced the smallest percent decrease in growth. The results are displayed in Figure 5.14, and it was determined that:

- SHU still produced the largest average daily growth which was 118% greater than CPTW SHU, and 82% greater than M/Gro.
- The lowest growths were shown by CPTW Shower and by CPTW, and these were closely followed by Shower.
- The effect of urine was shown by SHU producing 865% more growth than Shower, and CPTW SHU producing 500% more than CPTW Shower.

Differences in average daily growth produced by the three treatments CPTW, Shower, and CPTW Shower were not statistically significant ($p = 0.568$). The three treatments SHU, M/Gro, and CPTW SHU again produced growth results with statistically significant differences between them ($p < 0.001$), however the difference in growth produced by M/Gro and CPTW SHU treatments was not statistically significant ($p = 0.204$).

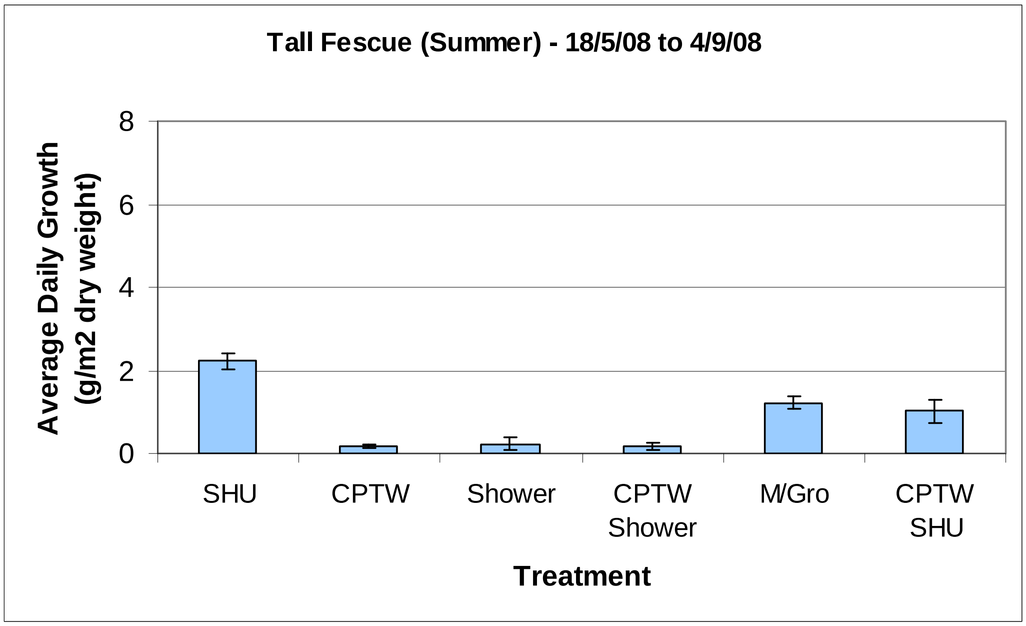

Figure 5.14 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 18/5/08 to 4/9/08 (Error bars indicate one standard deviation)

The following two photographs (Figure 5.15 and Figure 5.16) show the six Tall Fescue (Summer) samples from the F Set which had 108 days of growth from late Autumn to the start of Spring. It can be seen that the samples treated with CPTW (F4), Shower (F6), and CPTW Shower (F8) produced turf of very low growth and a somewhat dried out colour appearance than the samples treated with SHU (F2), M/Gro (F10), and CPTW SHU (F12). Similar results occurred with the Kikuyu samples that received the same treatments, which can be judged from the partly visible samples directly below in Figure 5.15, and directly above in Figure 5.16.

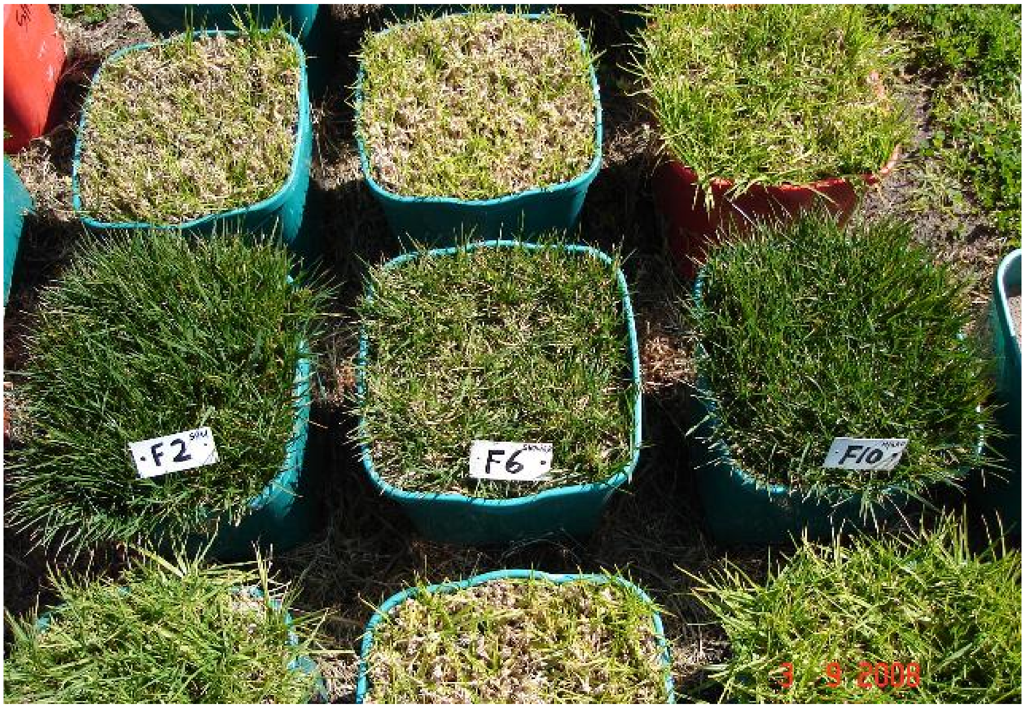

Figure 5.15 – Tall Fescue (Summer) samples that received SHU (F2), Shower (F6), and M/Gro (F10) treatments

Figure 5.16 – Tall Fescue (Summer) samples that received CPTW (F4), CPTW Shower (F8), and CPTW SHU (F12) treatments

5.3.5 Early Spring: Growth period 3/9/08 to 8/10/08

During this 5 week period in early spring, and compared to the previous mainly winter period, the average daily growth produced by all six treatments had increased. M/Gro continued its growth climb against SHU and CPTW SHU. The results are shown in Figure 5.17 from which the following was determined:

- SHU still produced the largest average daily growth but it was now reduced to 28.1% greater than M/Gro ($p = 0.004$), and 78.7% greater than CPTW SHU.
- The lowest growth was due to CPTW Shower, and this was closely followed by CPTW.
- The effect of urine was reduced with SHU producing 374% more growth than Shower, and CPTW SHU producing 244% more than CPTW Shower.

As in the previous period the differences in average daily growth produced by the three treatments CPTW, Shower, and CPTW Shower were not statistically significant ($p = 0.650$). The three treatments containing urine or plant food i.e. SHU, M/Gro, and CPTW SHU again produced growth results which differed by statistically significant amounts ($p < 0.001$).

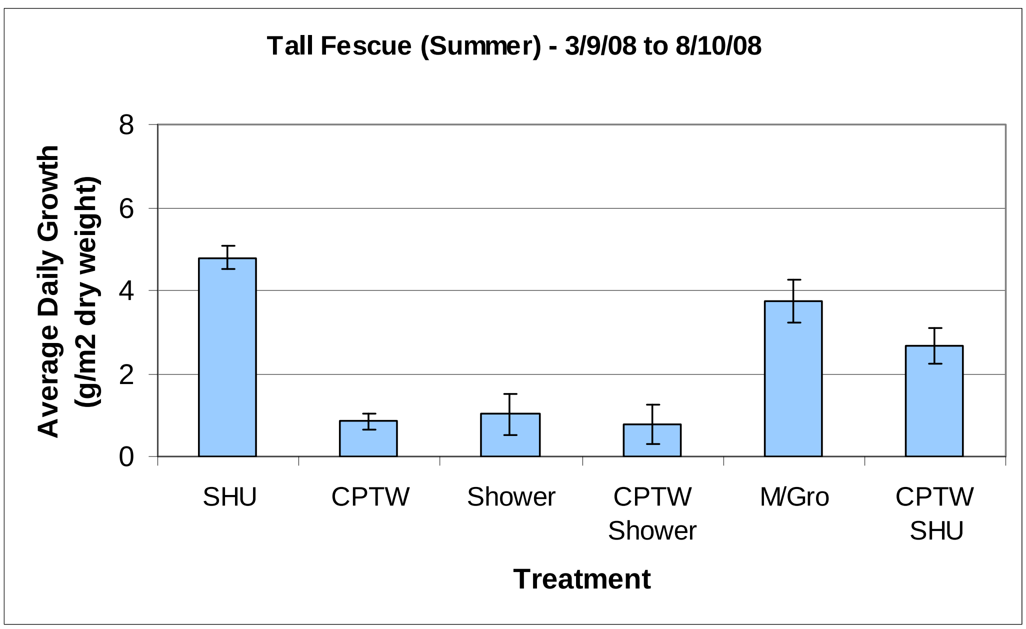

Figure 5.17 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 3/9/08 to 8/10/08 (Error bars indicate one standard deviation)

5.3.6 Spring: Growth period 5/10/08 to 20/11/08

Towards the end of this 6.5 week spring period the Tall Fescue samples sprouted flowering stalks, which ranged from 250 mm to 600 mm in height. The stalks were unevenly distributed throughout the samples making it difficult to draw conclusions from the data, which can be seen in Figure 5.18. Some of the flowering stalks grew around the edges away from the cutting area, but any stalk enclosed by the cutting frame was taken as part of the sample. After the cutting session on 20/11/08 the remaining stalks were removed and the experiment was continued, mainly to observe how the samples being treated with CPTW continued to perform.

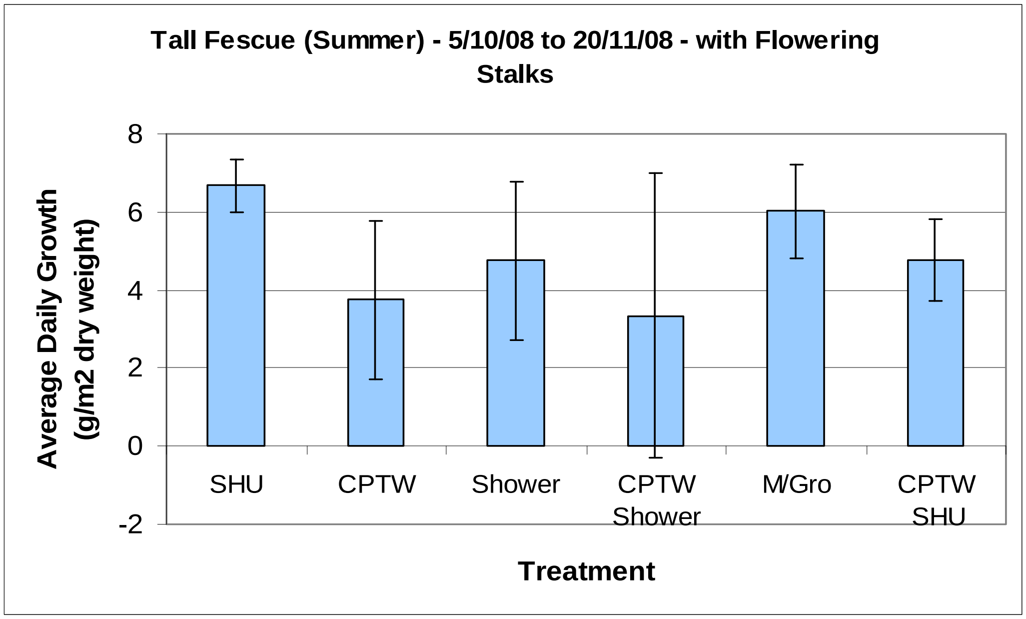

Figure 5.18 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer), including Flowering Stalks, over period 5/10/08 to 20/11/08 (Error bars indicate one standard deviation)

5.3.7 Late Spring and into Summer: Growth period 19/11/08 to 28/1/09

The results for this 11 week period are shown in Figure 5.19, and the following was determined:

- CPTW produced the lowest average daily growth.

- SHU continued to produce the largest growth which was approximately double that shown by M/Gro, and CPTW SHU treated samples.
- The effect of urine was still evident with SHU producing 437% more growth than Shower, and CPTW SHU showing 172% more than CPTW Shower.

As in the previous period the three treatments without added urine or plant food i.e. CPTW, Shower, and CPTW Shower again produced not statistically significant differences in average daily growth ($p = 0.742$), while SHU, M/Gro, and CPTW SHU again produced statistically significant differences in growth between them ($p < 0.001$). The growth result difference between M/Gro and CPTW SHU however was not statistically significant ($p = 0.822$).

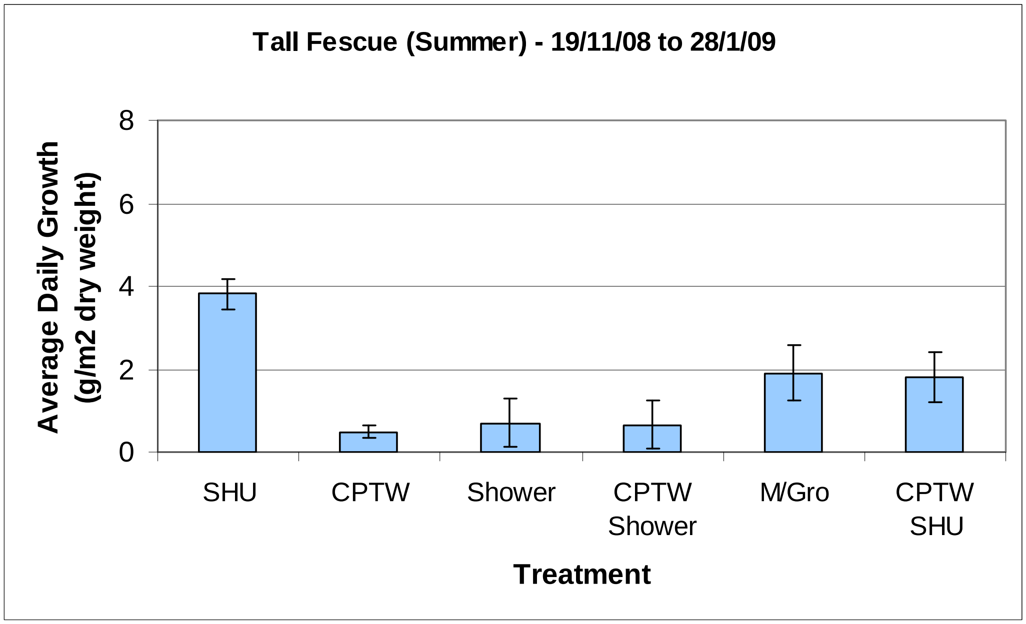

Figure 5.19 – Average Daily Growth (g/m² dry weight) of Tall Fescue (Summer) over period 19/11/08 to 28/1/09 (Error bars indicate one standard deviation)

5.4 Average Running Total Growth Heights of Tall Fescue

The secondary method of determining how the watering treatments affected growth of the Tall Fescue was to measure the growth height between each cutting session. Measurement of the growth height is a faster and simpler method than determining turf dry weights, however it does not take into account variations in the turf, such as grass thicknesses and variable densities of the turf sward, which are accounted for by determining dry weights of turf clippings.

5.4.1 Growth heights of Tall Fescue (Spring)

The experiment with Tall Fescue (Spring) ran for 332 days from 31/12/07 to 27/11/08, however for Day 332 only the heights of the blades of grass were measured, and not of the very irregular flowering stalks. The results are plotted on a single graph as running total growth heights (Figure 5.20), and as a reference for comparison a graph showing running total dry weights of turf clippings is provided (Figure 5.21). From 31/7/08 CPTW was converted to CPTWU with the addition of 1% v/v urine to encourage growth, therefore the code CPTW/U is used to signify both CPTW and CPTWU in the relevant Figures.

The following comments are based on results shown in Figure 5.20 up to 12/10/08 (Day 286), i.e. before the growth of the flowering stalks. It can be determined that:

- SHU produced significantly more average growth height through the whole experimental period than all the other treatments.
- The effect of urine was shown by SHU and SHU/5 respectively producing 210% and 36% more average total growth height than Shower, and by CPTW/U showing increased growth height after the addition of urine.
- The effect of the addition of nutrients as plant food was shown by M/Gro producing 129% more total growth height than Water.
- The least height was produced by Water followed by the deep rinses CPDR and ECDR i.e. the dilute greywater treatments.
- Shower produced 36.4% more total growth height than Water.
- Total wash ECTW produced 11.4% more growth height than deep rinse ECDR.
- Up to 10/7/08 total wash CPTW produced 29.2% more growth height than deep rinse CPDR.

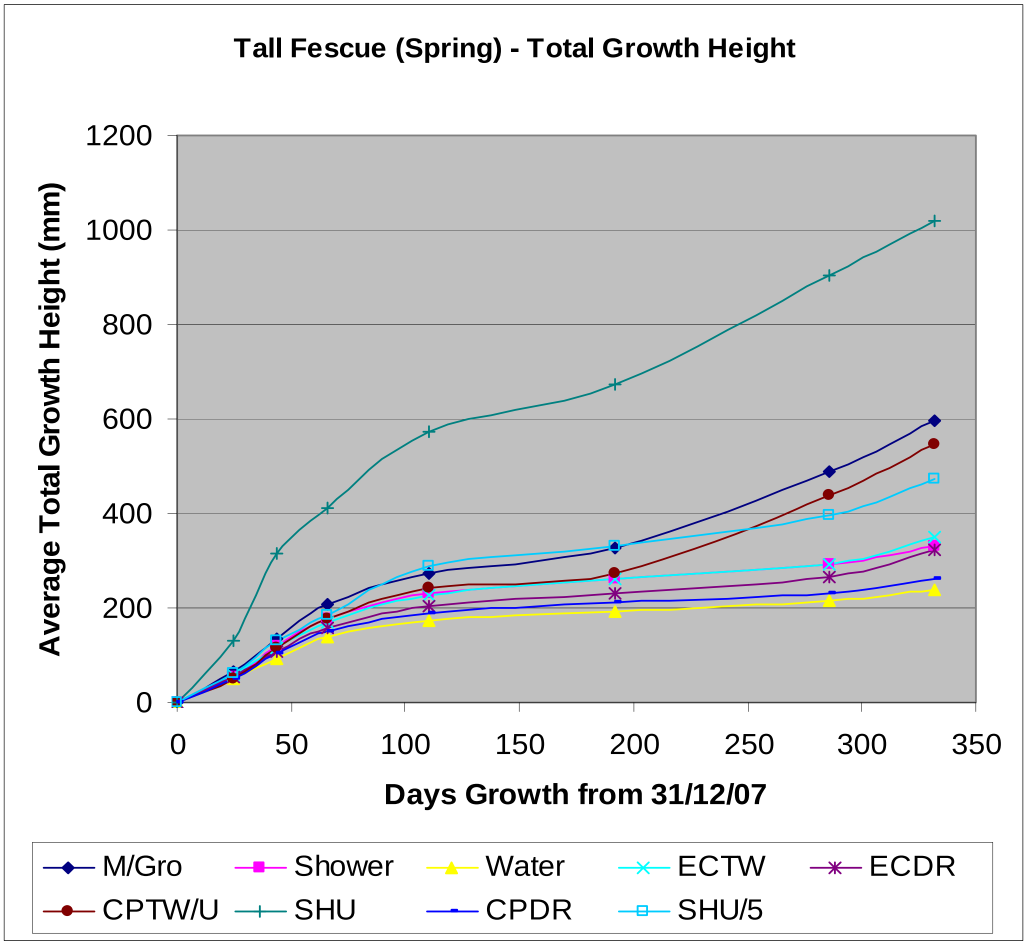

Figure 5.20 – Average Running Total Growth Heights (mm) of Tall Fescue (Spring) for each Treatment, over period 31/12/07 to 27/11/08

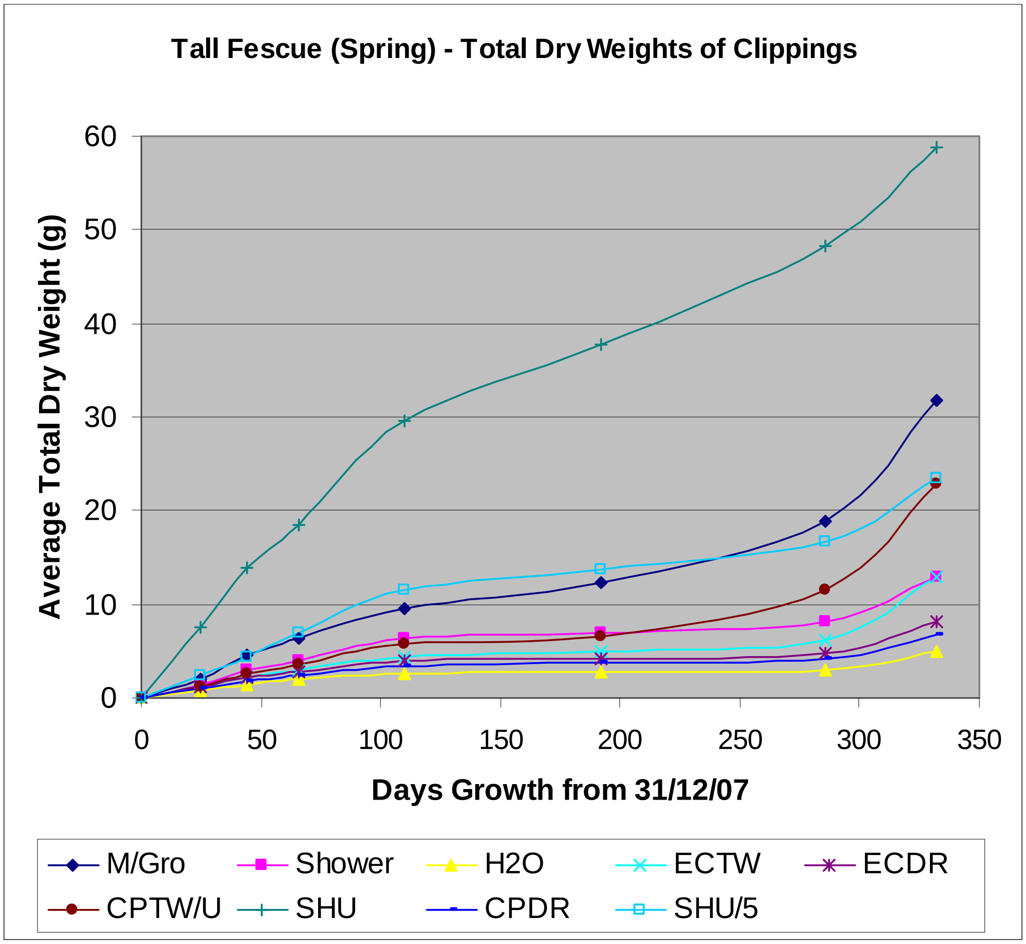

Figure 5.21 – Average Running Total Dry Weights (g) of Tall Fescue (Spring) clippings collected for each Treatment over growth period 31/12/07 to 27/11/08

It appears from the Tall Fescue (Spring) results the simple method of measuring the tallest growth heights may be useful to give acceptable results for assessing Tall Fescue growth, especially when it is not feasible to determine the dry weights of clippings. The graph showing running total growth heights is similar but not identical to the graph showing the running total dry weights of clippings. Both graphs show Water as the least contributor to long term turf grow followed by CPDR, ECDR, ECTW and Shower. Both also show SHU as the largest contributor to growth followed by the other treatments containing nutrients M/Gro, SHU/5, and CPTWU.

5.4.2 Growth heights of Tall Fescue (Summer)

The experiment with Tall Fescue (Summer) ran for 391 days from 3/1/08 to 28/1/09, The height measurements shown for Day 322 were only for the blades of grass and did not include the irregular large heights of the flowering stalks; therefore the increased slopes of the plots in Figure 5.22 after Day 245 (4/9/08) resulted mainly from spring growth and not from the flowering stalks. The results are plotted on a single graph as running total growth heights (Figure 5.22), and as a reference for comparison a graph showing running total dry weights of turf clippings is provided (Figure 5.23).

From the height measurement results it was deduced that:

- The largest average total growth height was produced by SHU, followed by CPTW SHU, M/Gro, Shower, CPTW, and CPTW Shower.
- The average growth heights produced by the three treatments Shower, CPTW, and CPTW Shower were basically similar.
- CPTW SHU produced greater growth height than M/Gro for most of the period, however by Day 391 M/Gro almost caught up to CPTW SHU.
- The effect of urine on turf growth height was shown by SHU producing 82.8% more height than Shower, and CPTW SHU producing 51.6% more than CPTW Shower.

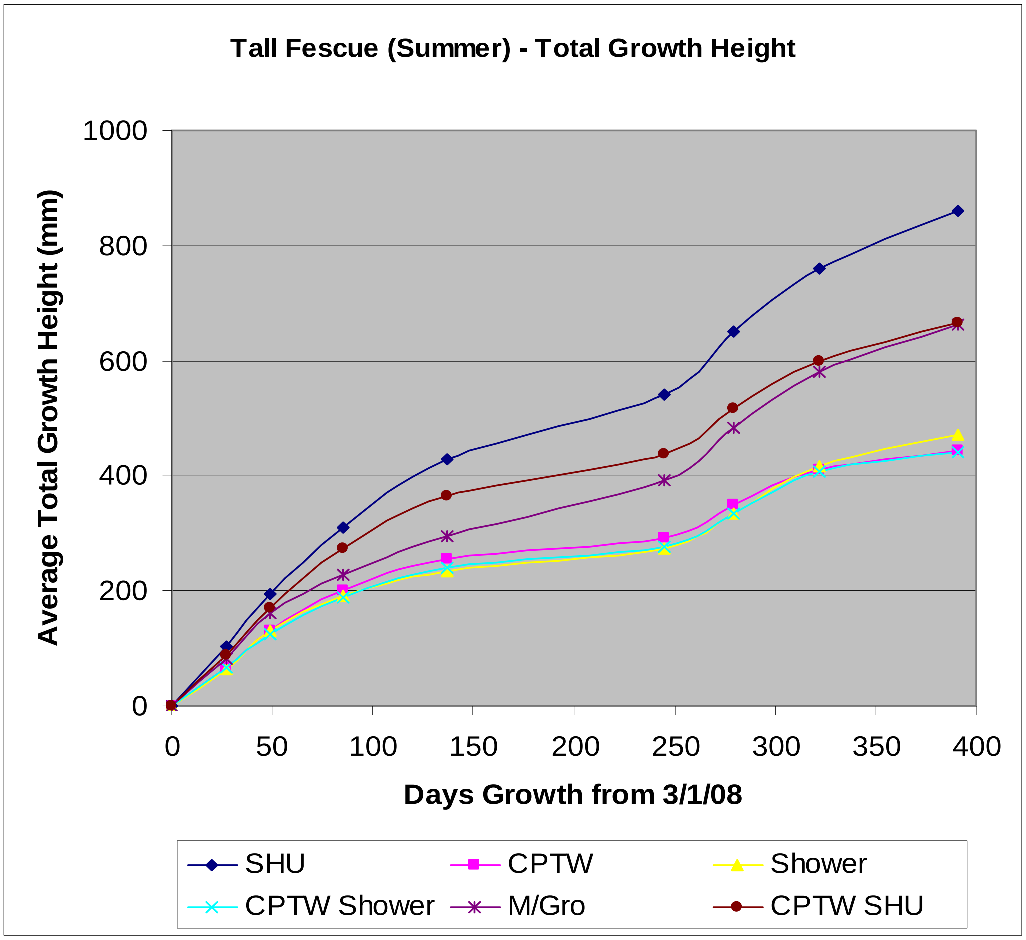

Figure 5.22 – Average Running Total Growth Heights (mm) of Tall Fescue (Summer) samples, resulting from each Treatment, over the period 3/1/08 to 28/1/09

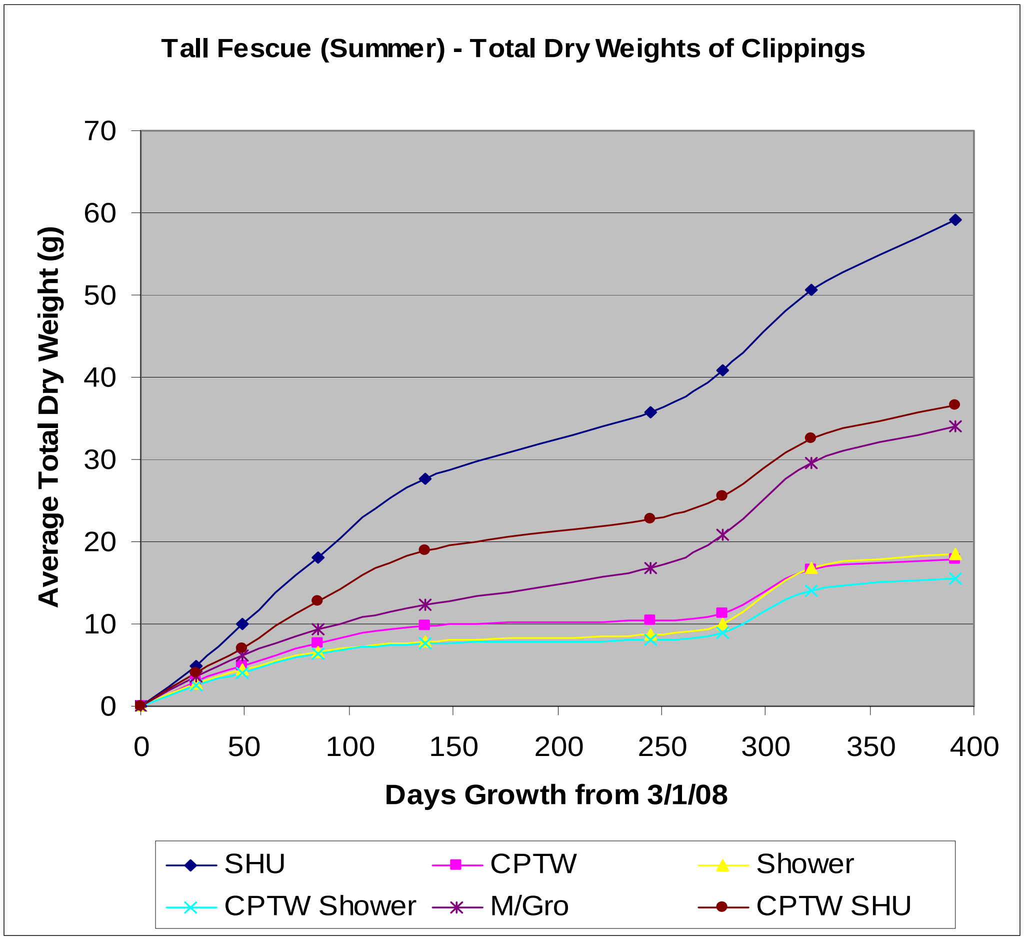

Figure 5.23 – Average Running Total Dry Weights (g) of Tall Fescue (Summer) clippings collected for each Treatment over growth period 3/1/08 to 28/1/09

The Tall Fescue (Summer) results confirm that the simple method of measuring the largest turf heights can be used to give acceptable results for Tall Fescue growth. Both the running total growth heights and the running total dry weights graphs are similar, and show that SHU is the greatest contributor to Tall Fescue growth, followed by CPTW SHU and M/Gro. The treatments that produced the least overall growth did not contain added urine or plant nutrients i.e. Shower, CPTW Shower, and CPTW.

5.5 Urine addition to poor growing Tall Fescue (Spring)

The aim of this experiment was to determine how the turf samples that were showing poor growth after more than a year of watering with the five lower nutrient treatments; Water, Shower, ECDR, ECTW, and CPDR, would respond if 0.5% v/v urine was added to the treatments. Any significant response would be a further indication that these water types lacked sufficient nutrients for sustaining growth, and that addition of urine was able to relieve this nutrient limitation. It had already been seen that CPTWU which contained 1% v/v urine from 31/7/08 to 11/10/08 eventually produced well growing and deep green coloured Tall Fescue turf. The samples that were previously treated with M/Gro and CPTWU were included in the experiment for comparison, and all the treatments containing 0.5% v/v urine had ‘0.5U’ added to their label codes, including CPTWU which from 12/10/08 had the urine content reduced to 0.5% v/v. It was decided not to follow the precedent established with SHU/5, such as to label Shower 0.5U as SHU/2, so that the names of the previous treatments applied to the samples could be retained in the label codes.

The experiment was commenced nine days after concluding the experiment on the Tall Fescue (Spring) samples, and was conducted on the same turf samples. The samples however were first subjected to soil core sampling, and also had the flowering stalks removed or cut at ground level. The experiment ran for 96 days over the summer months from 6/12/08 to 12/3/09 and involved two cutting sessions, the first over the three days 9/2/09 to 11/2/09, and the second was completed on 12/3/09.

The effect of adding 0.5% v/v urine to the five treatments was evident by the first cutting session at 65 days after starting the new treatments, when the urine containing treatments were producing increased growth and deeper green colouration. These effects were confirmed by the results obtained for the second growth period 9/2/09 to 12/3/09, in which the previously poor growing Tall Fescue (Spring) samples produced significantly increased growth that ranged from 11.8% larger to 16.2% smaller than the average daily growth produced by M/Gro treatment.

From Figure 5.24 it can be determined that:

- The average daily growth produced by CPDR 0.5U, Shower 0.5U, and ECTW 0.5U, were respectively 1.5%, 8.7%, and 11.8% larger than due to M/Gro.
- The average daily growth produced by Water 0.5U, and ECDR 0.5U were respectively now only 16.2% and 14.9% smaller than produced by M/Gro.
- The largest average daily growth was produced by CPTWU 0.5U which was respectively 48.3% and 32.6% larger than due to M/Gro and ECTW 0.5U.

These results support those obtained previously, that addition of small amounts of urine to water or dilute wastewater treatments, can produce growth rates that are similar to, or greater than, produced by the addition of Miracle Gro® All Purpose plant food. Statistical analysis (see below) confirmed that there was little difference between these treatments.

It was not surprising that in this three month trial CPTWU 0.5U produced greater growth than the other treatments containing 0.5% v/v urine, because the samples receiving CPTWU 0.5U were subjected to urine for an extra four months, and initially at a higher concentration.

Statistical analysis was done on the growth results from the second period 9/2/09 to 12/3/09, because by this time the effect of adding 0.5% v/v to the treatments had more time to stabilise. The average daily growth result shown by CPTWU 0.5U treated samples was not included in the statistical analysis, because these samples were subjected to extra four months of urine treatment.

Initial statistical analysis shows that the differences between the average daily growth produced by the six treatments M/Gro, CPDR 0.5U, Shower 0.5U, Water 0.5U, ECTW 0.5U, and ECDR 0.5U were statistically significant ($p = 0.005$). If however M/Gro treatment is compared with the treatments which produced larger average daily growth than M/Gro i.e. CPDR 0.5U, Shower 0.5U, and ECTW 0.5U the differences were not statistically significant ($p = 0.241$). The differences were also not statistically significant ($p = 0.192$), when comparing M/Gro treatment with the treatments which produced lower average daily growth than M/Gro i.e. Water 0.5U, and ECDR 0.5U.

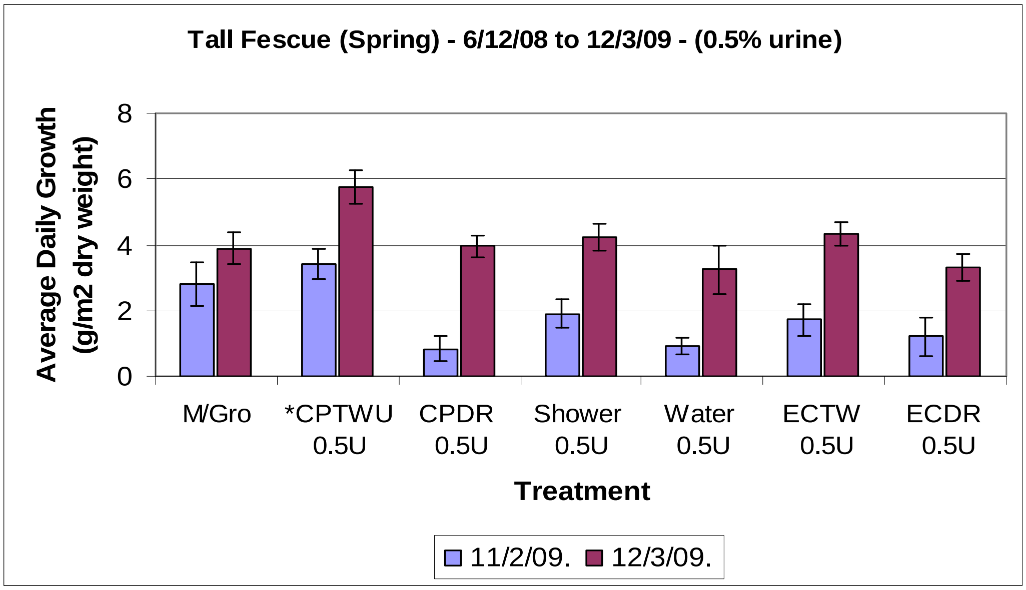

Figure 5.24 – Average Daily Growths (g/m² dry weight) of Tall Fescue (Spring) over the two periods 6/12/08 to 11/2/09 and 9/2/09 to 12/3/09 (Error bars indicate one standard deviation)

The final results of adding 0.5% v/v urine to the five treatments; Water, Shower, ECDR, ECTW, and CPDR can be seen in the photograph (Figure 5.25) of turf samples from the A Set. The even numbered Tall Fescue and the odd numbered Kikuyu samples had approximately four weeks of growth since their last cutting session. The five sets of treated samples; A3 and A4 (Shower 0.5U), A5 and A6 (Water 0.5U), A7 and A8 (ECTW 0.5U), A9 and A10 (ECDR 0.5U), plus A15 and A16 (CPDR 0.5U) show good four week growth and turf colour development when compared to the reference samples A1 and A2 (M/Gro), or A11 and A12 (CPTWU 0.5U). They also show good colour when compared to the samples G13 and G14, which had been treated with SHU/5 for more than fourteen months.

Figure 5.25 – Tall Fescue (Spring) and Kikuyu (Spring) – Effect of 0.5% v/v urine on previously poor growing Turf samples

Table 5.3 – Key to Figure 5.25

Tall Fescue **>**	A4 Shower 0.5U	A8 ECTW 0.5U	A12 CPTWU 0.5U	A16 CPDR 0.5U
Kikuyu **>**	A3 Shower 0.5U	A7 ECTW 0.5U	A11 CPTWU 0.5U	A15 CPDR 0.5U
Tall Fescue **>**	A2 M/Gro	A6 Water 0.5U	A10 ECDR 0.5U	G14 SHU/5
Kikuyu **>**	A1 M/Gro	A5 Water 0.5U	A9 ECDR 0.5U	G13 SHU/5

The following photograph (Figure 5.26) shows how the Tall Fescue samples A2 (M/Gro) and A6 (Water) looked like after the flowering stalks were removed, a few days before the start of the 0.5% v/v urine addition experiment.

Figure 5.26 – Appearance of Tall Fescue (Spring) samples that were receiving M/Gro (A2) and Water (A6) treatments – After removal of Flowering Stalks

5.6 Increased watering of SHU treated Tall Fescue (Spring)

During the previous summer it was observed that some Tall Fescue turf samples treated with SHU could suffer if watering with the standard 300ml was missed out or delayed in very hot weather. The problem was controlled by occasionally doubling up the watering level of all Tall Fescue samples to 600 ml i.e. whenever very hot weather was expected or if watering could not be done the next day. It was therefore decided to determine whether Tall Fescue samples which had been subjected to urine for more than a year, would require less vigilance during hot weather if 600ml of SHU (which contained 6 ml of urine) was applied at every watering session.

The experiment ran for 5 weeks from 25/12/08 to 31/1/09 and was conducted on the Tall Fescue (Spring) samples which had received SHU treatment from 9/11/07, and had been subjected to sample coring and removal of seed stalks before the experiment. The samples successfully survived five days of maximum temperatures ranging from 34.9º C to 41.1º C in the fortnight between 13/1/09 and 27/1/09, and also survived a massive three day heatwave of maximum temperatures 43.4º C, 44.3º

C, and 44.2º C recorded in Melbourne on 28, 29, and 30/1/09 respectively. This was the first time three consecutive days of temperatures above 43º C occurred in the history of record taking in Melbourne (Bureau of Meteorology 2009a). It was considered that the three day heatwave was a sufficient enough test and so the experiment was ended. The heat wave was sufficient to scorch some of the leaves of a few small trees growing nearby. It was not foreseen that a more severe test would be available seven days later, when a maximum temperature of 47.3º C was recorded at Essendon Airport some 8 km away to the North East (Bureau of Meteorology 2009b).

All five replicates grew well and withstood the hot drying conditions. The results are displayed in Figure 5.27 from which it can be seen that there was a reasonable consistency in the daily growth determined for the five replicate samples over the five week period. The difference between the highest and lowest daily growth of the Tall Fescue samples was 17.9%.

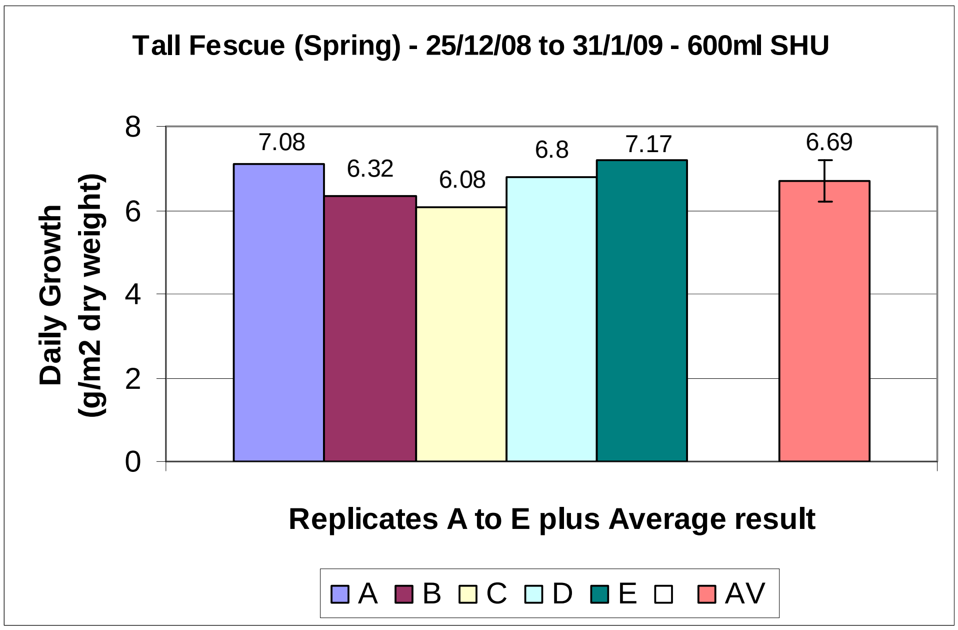

Figure 5.27 – Daily Growth (g/m² dry weight) per Pot of Tall Fescue (Spring) samples receiving 600ml of SHU each watering session between 25/12/08 and 31/1/09 (Error bar indicates one standard deviation)

The following Chapter 6 details the results of using greywaters on Kikuyu turf in the same way as for Tall Fescue in this chapter.

Chapter 6: Results of using Greywaters on Kikuyu turf

6.1 Introduction

The experiments on spring and summer planted Kikuyu turf were run at the same time as the experiments on Tall Fescue turf, and the same treatments as outlined in Chapter 4 were applied to the Kikuyu samples. The Spring and Summer planted samples of Kikuyu were randomised among the Tall Fescue samples in the same pot groupings, and the growth of the Kikuyu samples was evaluated by the same methods as described for the Tall Fescue samples. Statistical analysis of results was performed by the same methods as for the Tall Fescue samples (See Section 5.1.1), and the results are presented in the same way as for Chapter 5.

Although the first Kikuyu samples were planted into pots in spring the experiment could not be commenced until well into summer. The Kikuyu as supplied was over sown with Rye grass that ended up being unevenly distributed between the pots. The Rye grass was pulled out early on, and the spreading Kikuyu was allowed to repair itself within the pots. When this experiment commenced on 16/1/08 all the pots containing the Kikuyu (Spring) turf samples appeared to have uniform growth.

6.2 Average Daily Growth Rates of Kikuyu (Spring)

The results presented in the following Figure 6.1 to Figure 6.6 cover the 327 days of growth from 16/1/08 to 8/12/08. The results are presented in graphical form as Figures to make it easier to quickly see the growth differences. If required by the reader, the corresponding numerical data in Table form can be obtained by contacting the author. The experiment was ended on 8/12/08 because the samples treated with Water or the greywaters without added urine had been showing poor growth for a long time, probably due to a lack of plant nutrients. The samples with poor growth rates were later used in another short experiment.

6.2.1 Summer: Growth period 16/1/08 to 8/2/08

The results for this growth period are shown in Figure 6.1. From the results it was deduced that the overall pattern of responses reported in the previous Chapter for Tall Fescue also held true for Kikuyu, for this sampling period and across the whole experimental period. That is, relative to water control treatment, shower water and deep rinse water from the washing machine produced equivalent growth or small increases in growth. In comparison total wash treatments sometimes showed somewhat higher levels of growth. Greywater and water to which urine was added showed large increases in growth, sometime higher than the Miracle Gro nutrient addition control. In particular, for this growth period:

- Shower produced 80.6% more growth than Water.
- SHU produced the largest average daily growth which was respectively 52.8% and 212% larger than due to the next two highest M/Gro and SHU/5.
- The effect of plant food was shown by M/Gro producing 551% more growth than Water which also produced the lowest growth.
- The effect of urine was demonstrated by SHU and SHU/5 respectively producing 451% and 76.8% more growth than Shower.
- Total wash CPTW produced 32.9% more growth than the deep rinse CPDR. Result not statistically significant ($p = 0.253$).
- Deep rinse ECDR produced 27.9% more growth than total wash ECTW. Result not statistically significant ($p = 0.401$).
- The highest average daily growth from greywaters without added urine was due to CPTW.
- The phosphate based CPTW averaged 56.6% more growth than the non-phosphate based ECTW. Result not statistically significant ($p = 0.116$).

Differences between the average daily growth results produced by the six treatments without added urine or plant food i.e. Water, Shower, ECTW, ECDR, CPTW, and CPDR, were not statistically significant ($p = 0.090$). Statistically significant differences in growth however resulted between the three treatments which contained added urine or plant food i.e. SHU, SHU/5, and M/Gro ($p < 0.001$).

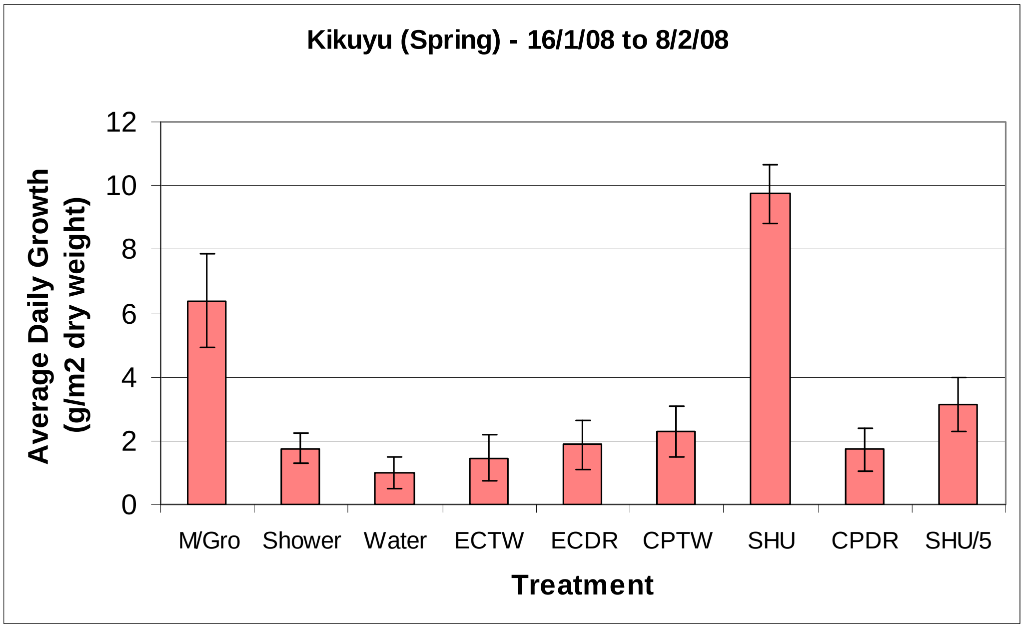

Figure 6.1 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over period 16/1/08 to 8/2/08 (Error bars indicate one standard deviation)

6.2.2 End of Summer: Growth period 6/2/08 to 3/3/08

The results for this 3.5 week growth period are shown in Figure 6.2, and compared to the previous period the average daily growths resulting from SHU and M/Gro treatments had decreased, whereas growth due to SHU/5 had increased. These results also show that:

- Shower produced 62% more growth than Water.
- SHU still had the largest growth producing 51% more than SHU/5 and 51.7% more than M/Gro.
- SHU and SHU/5 respectively produced 214% and 108% more growth than Shower.
- M/Gro had 236% more growth than Water – which again produced the lowest result.
- Total wash CPTW produced 40% more growth than deep rinse CPDR. Result not statistically significant ($p = 0.135$).
- Deep rinse ECDR produced 23.3% more growth than total wash ECTW. Result not statistically significant ($p = 0.347$).

- Phosphate containing CPTW produced 46.0% more growth than phosphate free ECTW. Result not statistically significant ($p = 0.150$).

The six treatments Water, Shower, ECTW, ECDR, CPTW, and CPDR again produced average daily growths with differences between them that were not statistically significant ($p = 0.104$). The three treatments SHU, SHU/5, and M/Gro again produced growth results with statistically significant differences between them ($p = 0.001$). However the average daily growth results produced by SHU/5 and M/Gro treatments were similar ($p = 0.943$).

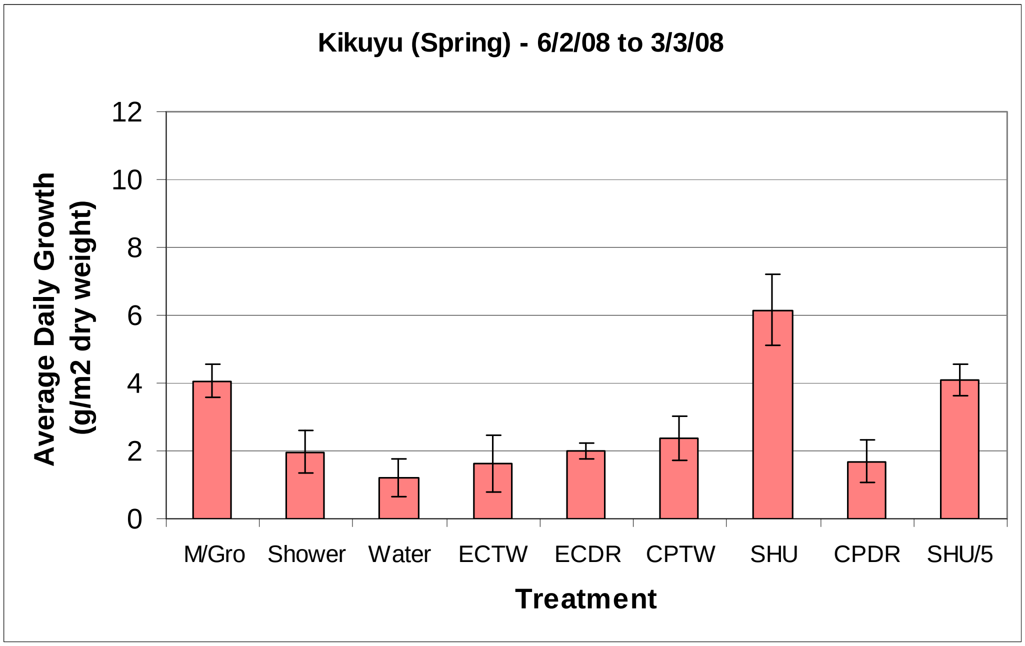

Figure 6.2 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over period 6/2/08 to 3/3/08 (Error bars indicate one standard deviation)

6.2.3 Early Autumn: Growth period 29/2/08 to 16/4/08

During this 6.5 week period all the treatments except SHU/5 produced increased average daily growth, when compared against the previous end of summer period. The growth results are shown in Figure 6.3 from which it was determined that:

- Shower produced 94% more growth than Water.

- The largest growth was again due to SHU producing 64.8% more than the next highest M/Gro, and 129% more than the third highest SHU/5.
- SHU and SHU/5 respectively produced 147% and a small 8% more growth than Shower.
- M/Gro produced 191% more growth than Water which again had the lowest growth.
- The largest average daily growth from greywaters without added urine was due to Shower, which was only 1.9% larger than the next highest CPTW.
- Total wash CPTW produced 33.1% more growth than deep rinse CPDR. Result this time was statistically significant ($p = 0.001$).
- Total wash ECTW produced 6.1% more growth than deep rinse ECDR. Result not statistically significant ($p = 0.604$).
- Phosphate containing CPTW produced 8.2% more growth than phosphate free ECTW. Result not statistically significant ($p = 0.429$).

The five treatments Shower, ECTW, ECDR, CPTW, and the 0.2% v/v urine containing SHU/5 produced average daily growths with differences between them that were not statistically significant ($p = 0.138$). Inclusion of Water and CPDR growth results with Shower, ECTW, ECDR, and CPTW resulted in statistically significant differences in growth between all six treatments ($p = <0.001$). The three treatments containing plant food or urine i.e. SHU, SHU/5, and M/Gro, again produced growth results with statistically significant differences between them ($p < 0.001$).

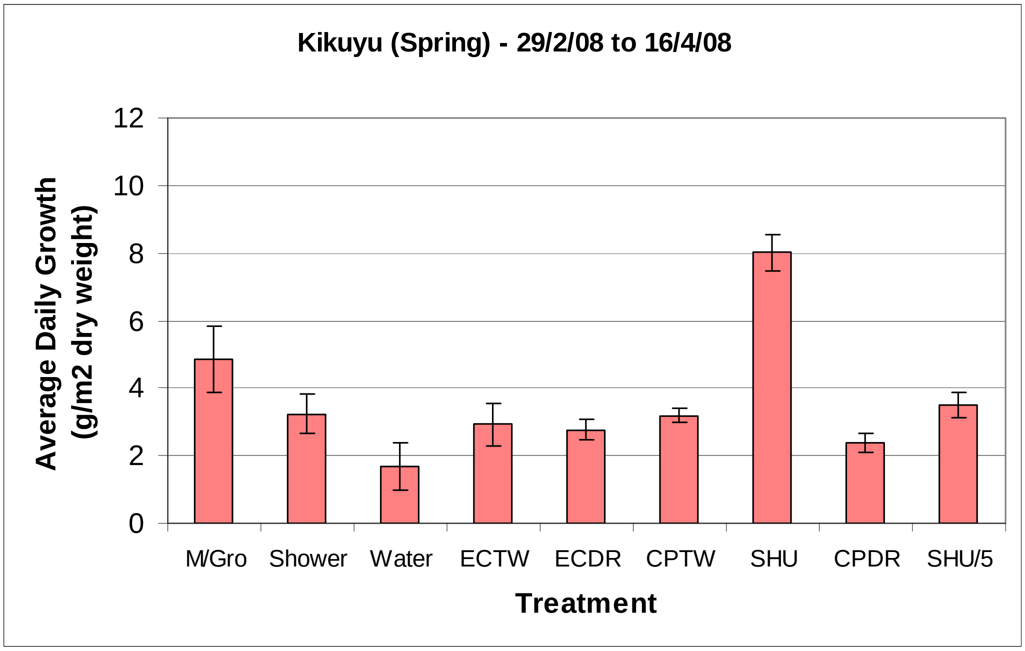

Figure 6.3 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over period 29/2/08 to 16/4/08 (Error bars indicate one standard deviation)

6.2.4 Mid Autumn to Mid Winter: Growth period 11/4/08 to 15/7/08

During this 13.5 week period the average daily growth results produced by all nine treatments reduced significantly compared to the previous early autumn period. The growth results are shown in Figure 6.4, and show that:

- Shower produced 145% more growth than Water.
- M/Gro now produced the largest growth which was 20.7% more than for SHU, and 169% more than for the third largest SHU/5.
- The effect of plant food was still evident with M/Gro producing an average daily growth 695% greater than Water, which continued to produce the smallest growth.
- The effect of urine was shown by SHU producing 169% more growth than Shower, and SHU/5 producing 20.4% more growth than Shower. The latter result not statistically significant ($p = 0.448$).
- The largest average daily growth from greywaters without added urine was again due to Shower, which was 21.3% greater than the next largest ECTW.

- CPTW produced only 2.5% larger growth than CPDR, and ECTW produced 11.9% more than ECDR. Both results not statistically significant ($p = 0.897$ and 0.718 respectively).
- The phosphate based CPTW produced 14.6% more growth than the non-phosphate based ECTW. Result not statistically significant ($p = 0.621$).

The six treatments without added urine or plant food i.e. Shower, Water, ECTW, ECDR, CPTW, and CPDR produced average daily growths with differences between them that were not statistically significant ($p = 0.179$). The three treatments with added urine or plant food i.e. SHU, SHU/5, and M/Gro again produced growth results with statistically significant differences between them ($p = 0.014$), however the growth difference between SHU and M/Gro was not statistically significant ($p = 0.459$).

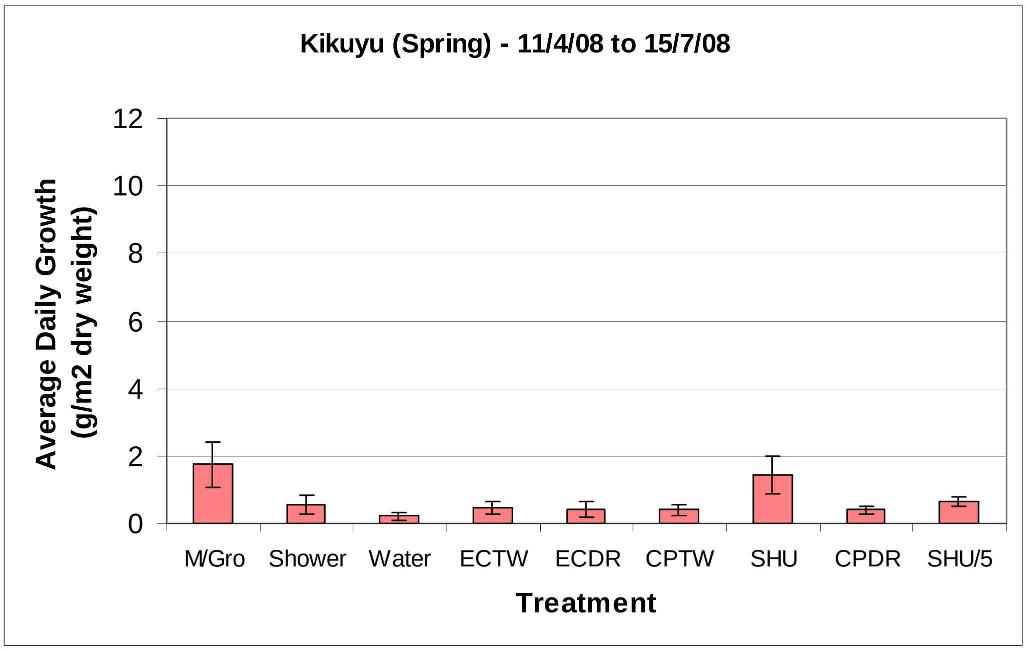

Figure 6.4 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over period 11/4/08 to 15/7/08 (Error bars indicate one standard deviation)

6.2.5 Mid Winter to Early Summer: Growth period 14/7/08 to 8/12/08

This growth period of 21 weeks was long because the growth produced by Water was almost non existent, and the growths produced by the greywaters without added urine were very low to enable earlier cutting of these samples. It appeared that the samples receiving treatments that did not contain added urine or plant food were lacking nutrients, and so 1% v/v urine was added to CPTW from 31/7/08 to determine whether growth could be stimulated by the addition of a plant nutrient source. The CPTW with the urine was relabelled as CPTWU (Cold Power Total Wash plus Urine). The samples treated with M/Gro, SHU, SHU/5, and CPTWU grew at a faster rate especially from mid Spring, resulting in the samples receiving these four treatments having an extra cut from 8/10/08 to 9/10/08. The dry weights of the clippings from the extra cut were included in the collected weights for the whole 21 weeks period.

The average daily growth results over the 21 week period are shown in Figure 6.5, and it was determined that:

- Shower produced 288% more growth than Water.
- M/Gro and SHU produced almost equal average daily growth, with M/Gro being 0.7% greater ($p = 0.967$).
- The third largest average daily growth was produced by CPTWU which had 162% more growth than the fourth largest SHU/5.
- M/Gro produced 3600% more growth than Water which again produced the lowest growth.
- The effect of urine was shown by SHU and SHU/5 respectively producing 848% and 148% more growth than Shower.
- Total wash ECTW produced 31.8% more growth than deep rinse ECDR. Result not statistically significant ($p = 0.470$).
- The highest average daily growth from greywaters without added urine was produced by CPDR, which was 19.4% greater than the next highest Shower.

The five treatments Water, Shower, ECTW, ECDR, and CPDR produced average daily growth results with differences between them that were not statistically significant (p = 0.461). The four treatments with added urine or plant food i.e. SHU, SHU/5, M/Gro, and CPTWU produced growth results with statistically significant differences between them ($p < 0.001$). However exclusion of SHU/5 (lowest) showed that the growth results due to SHU, M/Gro, and CPTWU did not differ statistically significantly (p = 0.101).

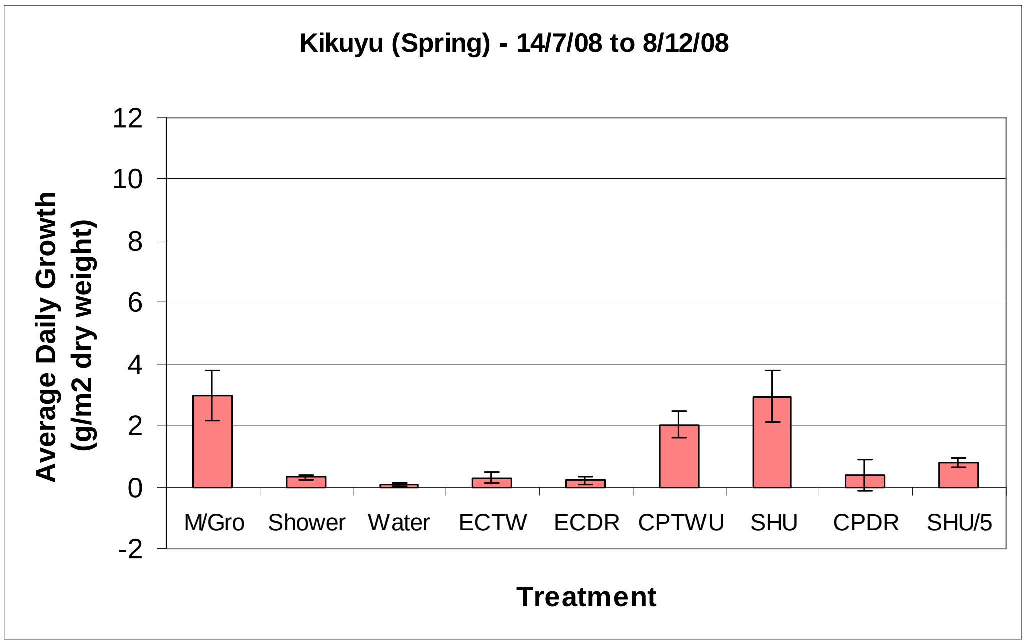

Figure 6.5 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over period 14/7/08 to 8/12/08 (Error bars indicate one standard deviation)

Most of the growth occurred towards the end of the 21 week period when the weather got warmer. Figure 6.6 shows the large differences in average daily growth results produced by the four treatments M/Gro, SHU, SHU/5, and CPTWU from before and after the extra cutting session on 8/10/08 to 9/10/08.

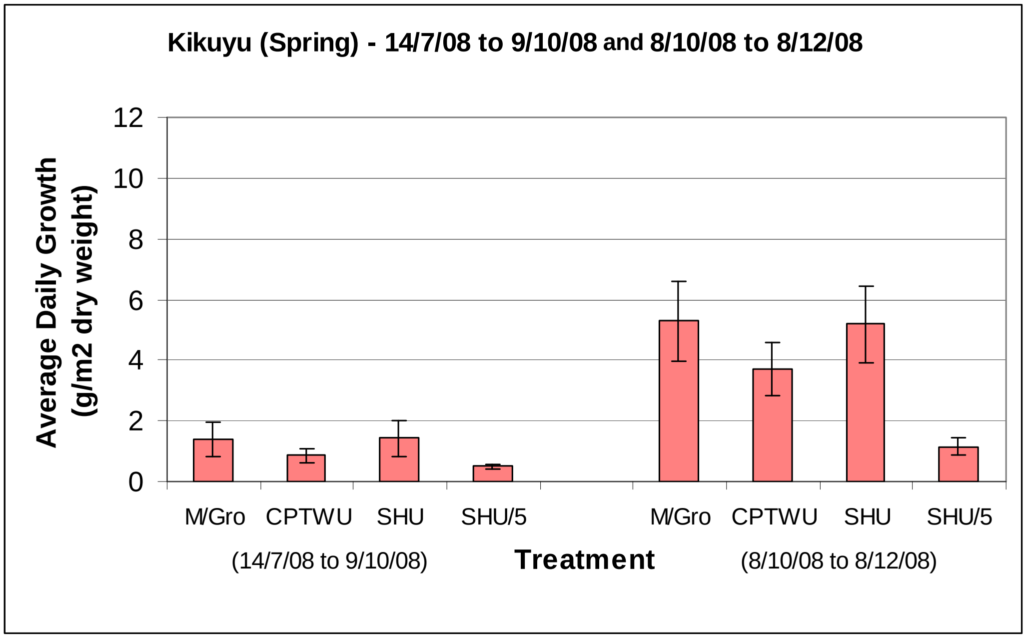

Figure 6.6 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over the two periods 14/7/08 to 9/10/08 and 8/10/08 to 8/12/08
(Error bars indicate one standard deviation)

6.3 Average Daily Growth Rates of Kikuyu (Summer)

As for the Tall Fescue (Summer) turf, this experiment investigated the growth of Kikuyu watered with blends of a phosphorus containing laundry water (CPTW), and with water from the shower (with and without urine). The two blends of greywaters CPTW SHU and CPTW Shower were compared against the component waters CPTW, SHU, and Shower, and against M/Gro treatment. The results presented below in Figures 6.7, 6.8, 6.9, 6.10, 6.13, and 6.14 cover six growth periods spanning 3/1/08 to 29/1/09.

6.3.1 Mid Summer: Growth period 3/1/08 to 3/2/08

The growth results for this initial 4.5 week period are shown in Figure 6.7, and it can be determined that:

- SHU produced the largest average daily growth which was 45.3% greater than M/Gro and 48.5% more than CPTW SHU.

- The effect of urine was already evident with SHU producing 89.4% more growth than Shower, and CPTW SHU producing 14.3% more than CPTW Shower. However the latter result was not statistically significant ($p = 0.130$).
- The smallest growth resulted from CPTW, followed by Shower.

The three treatments without added urine or plant food i.e. CPTW, Shower, and CPTW Shower, produced average daily growth results which did not differ by statistically significant amounts ($p = 0.294$). Inclusion of M/Gro with the above three treatments also resulted in growth differences between the four treatments that were not statistically significant ($p = 0.103$). The three treatments containing added urine or plant food i.e. SHU, M/Gro, and CPTW SHU produced growth results which differed by statistically significant amounts ($p = 0.002$), however the growth result between M/Gro and CPTW SHU was not statistically significant ($p = 0.867$).

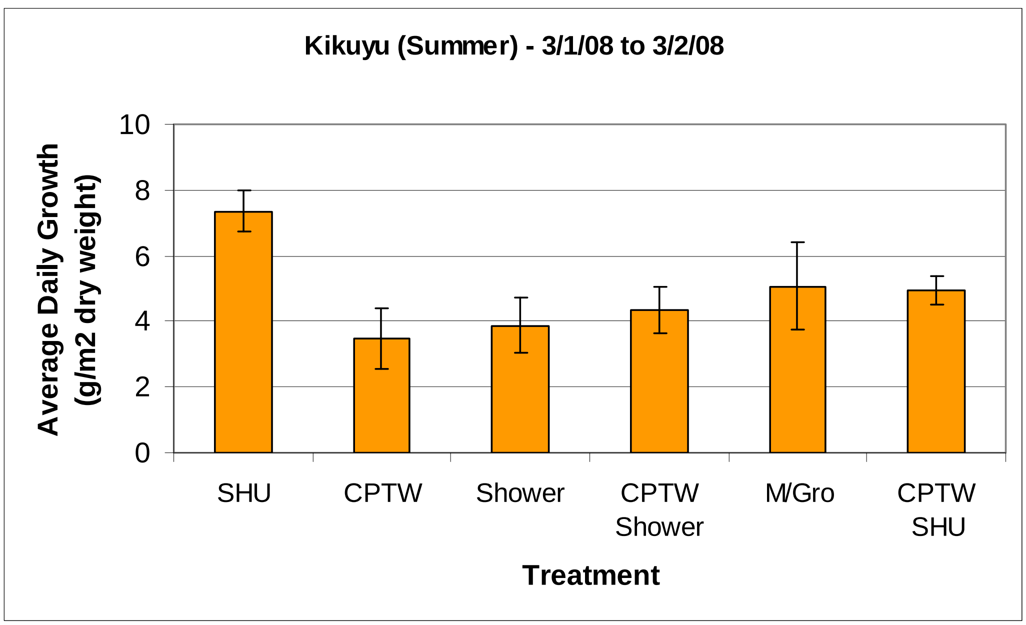

Figure 6.7 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 3/1/08 to 3/2/08 (Error bars indicate one standard deviation)

6.3.2 Late Summer to Early Autumn: Growth period 30/1/08 to 24/3/08

During this eight week period the average daily growth results of SHU and CPTW SHU treated samples slightly increased, whereas the samples treated with M/Gro and the three greywaters without added urine produced decreased growth. The results are shown in Figure 6.8, and it was determined that:

- SHU produced the largest average daily growth which was 37.2% more than CPTW SHU, and 84.5% more than M/Gro.
- The effect of urine resulted in SHU producing 208% more growth than Shower, and CPTW SHU producing 93% more than CPTW Shower.
- The smallest growth was due to Shower followed by CPTW Shower, and CPTW.

Unlike the previous period the three treatments Shower, CPTW, and CPTW Shower produced average daily growth results that differed by statistically significant amounts ($p = 0.024$), however the result between CPTW and CPTW Shower was not statistically significant ($p = 0.464$). The three treatments SHU, M/Gro, and CPTW SHU produced growth results with statistically significant differences between them ($p < 0.001$), however unlike the previous period the growth result difference between M/Gro and CPTW SHU was statistically significant ($p < 0.001$).

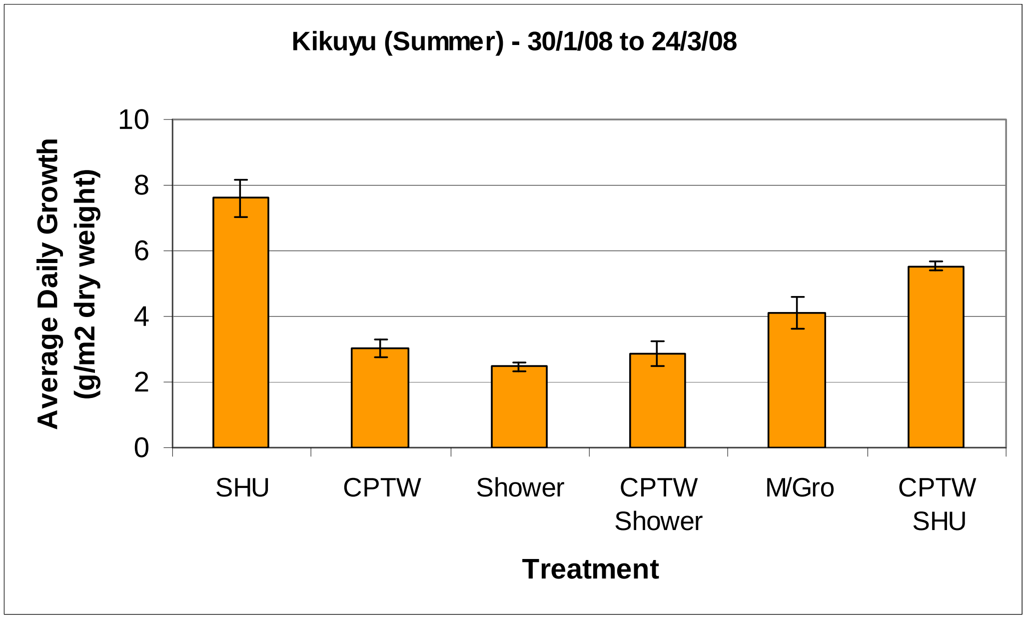

Figure 6.8 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 30/1/08 to 24/3/08 (Error bars indicate one Standard Deviation)

6.3.3 Autumn: Growth period 23/3/08 to 16/5/08

During this eight week period the average daily growth results produced by all six treatments had decreased significantly compared to the previous period. The results are shown in Figure 6.9, and it can be seen that:

- SHU still produced the largest average daily growth which was 25.5% more than CPTW SHU, and 28.3% more than M/Gro.
- The effect of urine is shown by SHU producing 228% more growth than Shower, and CPTW SHU producing 147% more than CPTW Shower.
- The smallest growth was again due to Shower, closely followed by CPTW Shower, and CPTW.

The three treatments CPTW, Shower, and CPTW Shower produced average daily growth results with differences that were not statistically significant ($p = 0.778$), and the three treatments with added urine or plant food i.e. SHU, M/Gro, and CPTW SHU this time also produced growth results which did not differ by statistically significant amounts ($p = 0.119$).

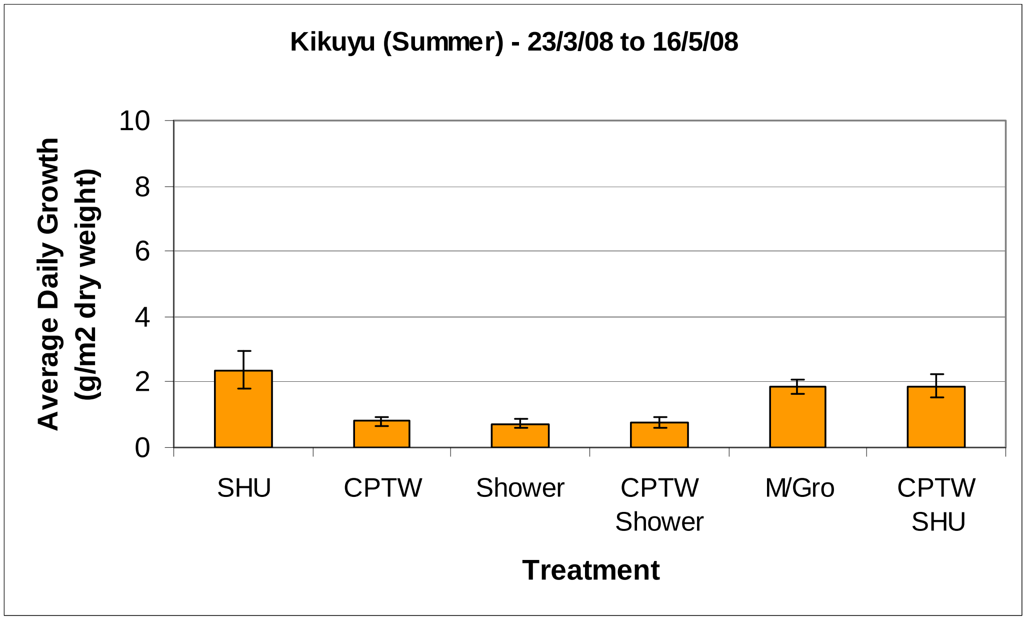

Figure 6.9 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 23/3/08 to 16/5/08 (Error bars indicate one standard deviation)

6.3.4 Late Autumn to Early Spring: Growth period 15/5/08 to 8/9/08

During this 16.5 week period over mainly cooler months the average daily growth results produced by all six treatments further decreased. The results are shown in Figure 6.10. It can be seen that:

- M/Gro now produced the largest average daily growth which was 20.8% more than SHU, and 118% more than CPTW SHU.
- The effect of urine was shown by SHU producing 621% more average growth than Shower, and CPTW SHU producing 331% more than CPTW Shower.
- The smallest growths resulted from the greywaters without added urine i.e. CPTW Shower, Shower, and CPTW, all of which produced similar results.

Similar to the previous period the growth differences produced by CPTW, Shower, and CPTW Shower were again not statistically significant ($p = 0.964$). The treatments SHU, M/Gro, and CPTW SHU again produced growth results with statistically significant differences between them ($p = 0.003$), however the difference in growth between SHU and M/Gro was not statistically significant ($p = 0.246$).

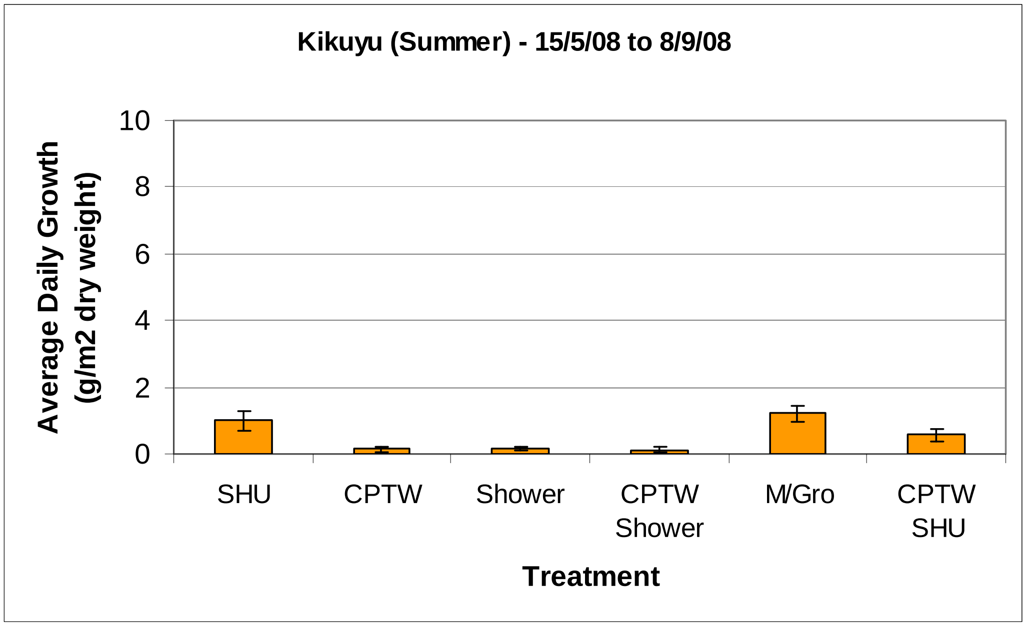

Figure 6.10 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 15/5/08 to 8/9/08 (Error bars indicate one standard deviation)

The following two photographs (Figure 6.11 and Figure 6.12) show the six Kikuyu (Summer) samples from the J Set which had 111 days of growth from late autumn to the start of spring. The samples treated with CPTW (J1), CPTW Shower (J5), and Shower (J7) produced turf of very low growth and a somewhat dried out colour appearance than the samples treated with SHU (J3), CPTW SHU (J9), and M/Gro (J11). J9 also shows less growth than J11 and a trace of dry coloured turf.

Figure 6.11 – Kikuyu (Summer) samples treated with CPTW (J1), CPTW Shower (J5), and CPTW SHU (J9)

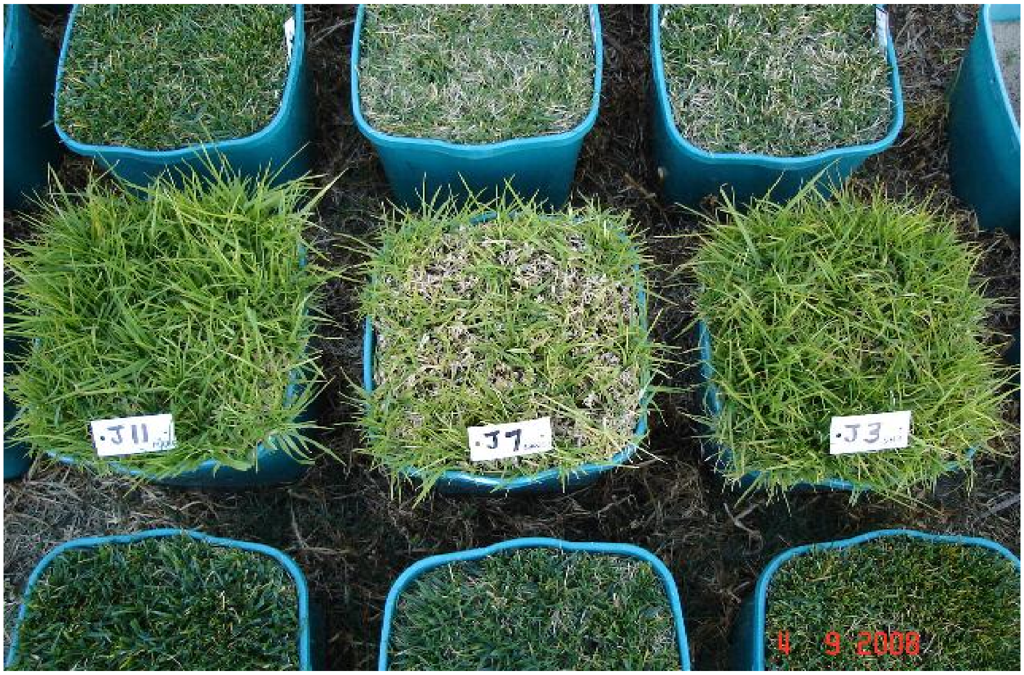

Figure 6.12 – Kikuyu (Summer) samples treated with SHU (J3), Shower (J7), and M/Gro (J11)

6.3.5 Early to Mid Spring: Growth period 4/9/08 to 14/10/08

During this six week spring period the average daily growth results produced by all six treatments increased compared to the previous period. The results are shown in Figure 6.13, and it was determined that:

- SHU now produced 5.5% more average daily growth than M/Gro, and 78.8% more than CPTW SHU.
- The effect of urine was shown by SHU producing 763% more growth than Shower, and CPTW SHU producing 320% more than CPTW Shower.
- The smallest growth was produced by Shower, closely followed by CPTW Shower, and CPTW.

The three treatments CPTW, Shower, and CPTW Shower again produced average daily growth results that did not differ by statistically significant amounts ($p = 0.576$), while the treatments containing urine or plant food i.e. SHU, M/Gro, and CPTW SHU again produced growth results with statistically significant differences between them ($p = 0.001$). However the difference in growth produced by SHU and M/Gro was not statistically significant ($p = 0.646$).

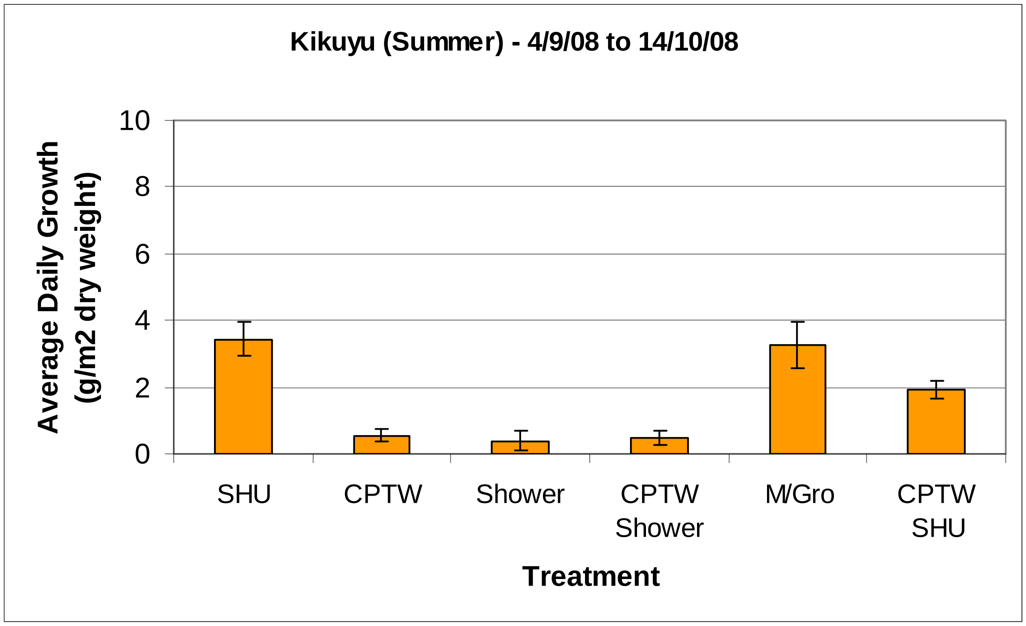

Figure 6.13 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 4/9/08 to 14/10/08 (Error bars indicate one Standard Deviation)

6.3.6 Mid Spring to End of Summer: Growth period 13/10/08 to 29/1/09

During this 15.5 week period the average daily growth results produced by SHU, CPTW SHU, and M/Gro increased significantly, whereas the average daily growth results produced by the greywaters without added urine (Shower, CPTW Shower, and CPTW), did not increase. CPTW did not produce the increased growth that the urine containing CPTWU did in the Kikuyu (Spring) samples. The samples treated with the three urine free greywaters were lacking plant nutrients. The results are shown in Figure 6.14, and it was determined that:

- SHU produced 21.5% more average daily growth than M/Gro, and 63.3% more than CPTW SHU.
- The effect of urine was shown by SHU producing 1579% more growth than Shower, and CPTW SHU producing 878% more than CPTW Shower.
- The smallest growth was due to Shower, closely followed by CPTW Shower, then CPTW.

The three treatments SHU, M/Gro, and CPTW SHU again produced average daily growth results with statistically significant differences between them ($p = 0.006$), however the difference in growth between SHU and M/Gro was again not statistically significant ($p = 0.128$). The growth results produced by CPTW, Shower, and CPTW Shower again did not differ by statistically significant amounts ($p = 0.617$).

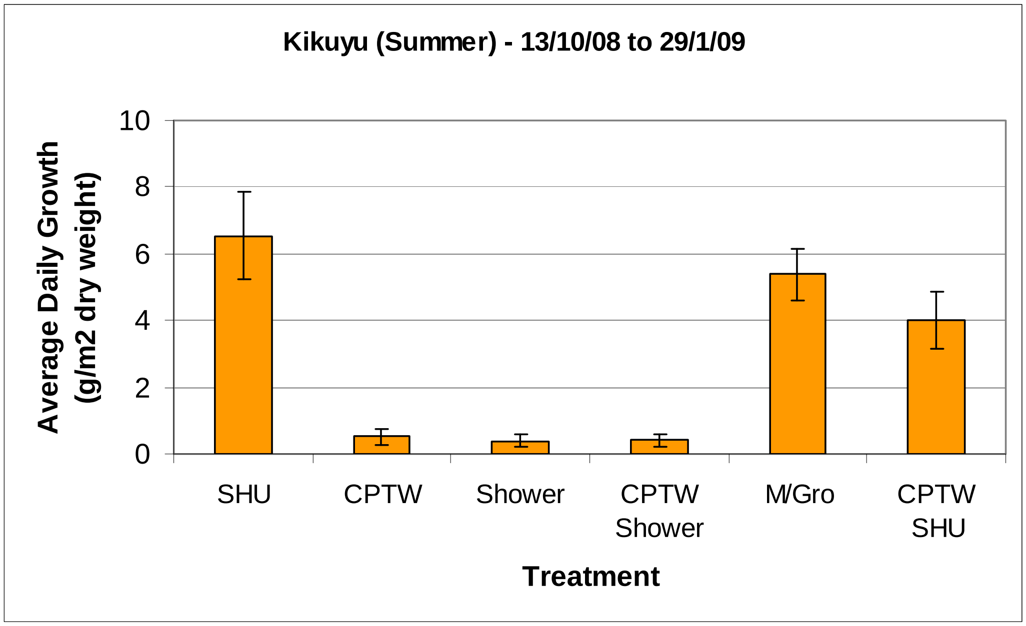

Figure 6.14 – Average Daily Growth (g/m² dry weight) of Kikuyu (Summer) over period 13/10/08 to 29/1/09 (Error bars indicate one standard deviation)

6.4 Average Running Total Growth Heights of Kikuyu

Determining the average largest height that the Kikuyu samples grew between each cutting session was the secondary method of determining how the watering treatments affected the growth. The growth heights produced by each of the treatments were determined from the differences in height measurements taken before and after cutting each sample. The height measurements taken before cutting of the Kikuyu were of the blades of grass extending above the runners and growing upwards, and not of the lengths of horizontal runners. Although the growth height method is faster and simpler than determining dry weights of turf clippings, it does not take into account variations in the turf, such as grass thicknesses and variable densities of the turf sward, which are accounted for by the dry weight method.

6.4.1 Growth heights of Kikuyu (Spring)

The experiment was run with Kikuyu (Spring) for 327 days from 16/1/08 to 8/12/08. As for the Tall Fescue the growth height measurements are plotted on a single graph as running total growth heights (Figure 6.15), and as a reference for comparison a graph showing the running total dry weights of Kikuyu clippings, which were obtained at the same times, is provided (Figure 6.16). From 31/7/08 CPTW was altered to CPTWU with the addition of 1% v/v urine, therefore CPTW/U is used to designate CPTW before 31/7/08 and CPTWU after that date.

The average running total growth heights of Kikuyu (Spring) up to the end of the experiment on Day 327, are shown in Figure 6.15. The results show that:

- The largest average total growth height throughout the period was shown by SHU, followed by M/Gro, and then SHU/5.
- The least growth height was shown by Water, followed by the deep rinses CPDR and ECDR.
- The effect of urine was shown by SHU and SHU/5 respectively producing 178% and 57.6% more growth height than Shower, and by CPTW/U showing increased growth after the addition of urine.
- The effect of plant food was shown by M/Gro producing 219% more total growth height than Water.
- Shower produced 46.7% more growth height than Water.
- Total wash ECTW produced 29.7% more growth height than ECDR.
- Up to 15/7/08 (Day 181) CPTW produced 21.3% more growth height than deep rinse CPDR.
- Of the greywaters without added urine ECTW produced the largest growth height. Up to 15/7/08 (Day 181) the growth height was 8.4% greater than due to Shower, and up to the end (Day 327) it was 8.6% greater.

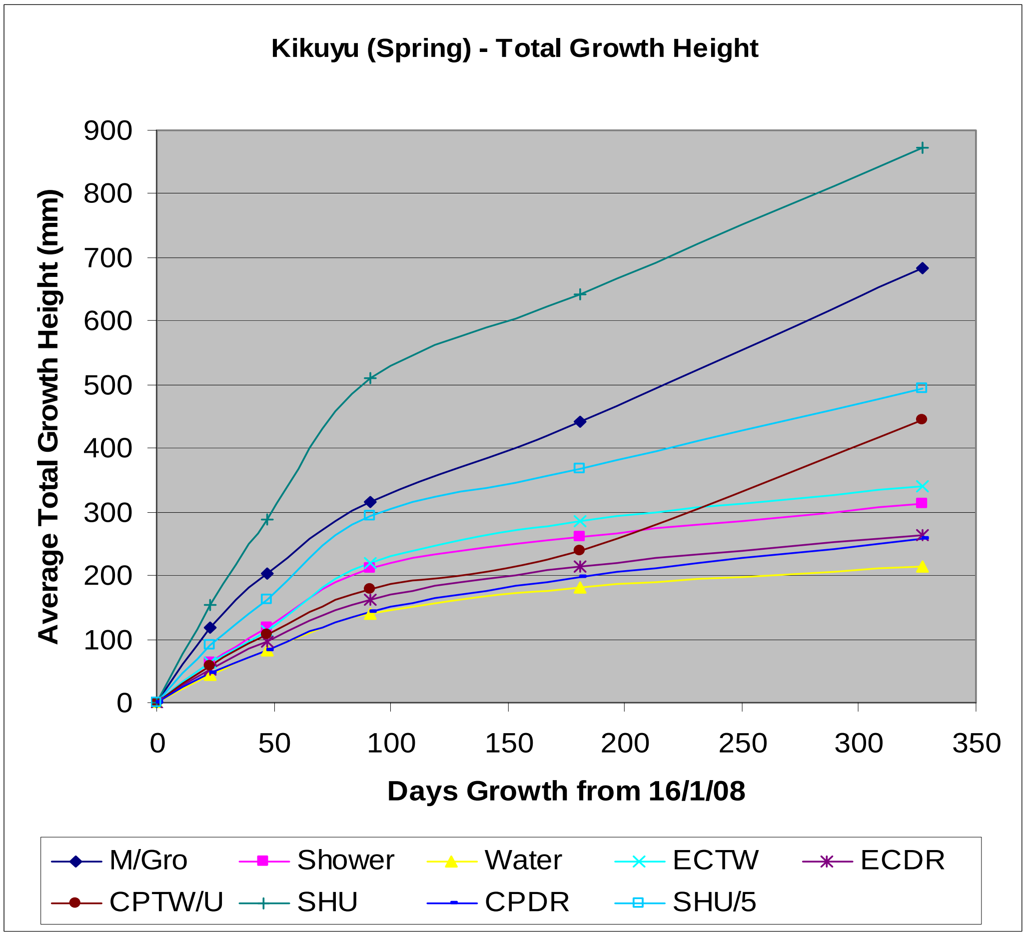

Figure 6.15 – Average Running Total Growth Heights (mm) of Kikuyu (Spring) for each Treatment, over period 16/1/08 to 8/12/08

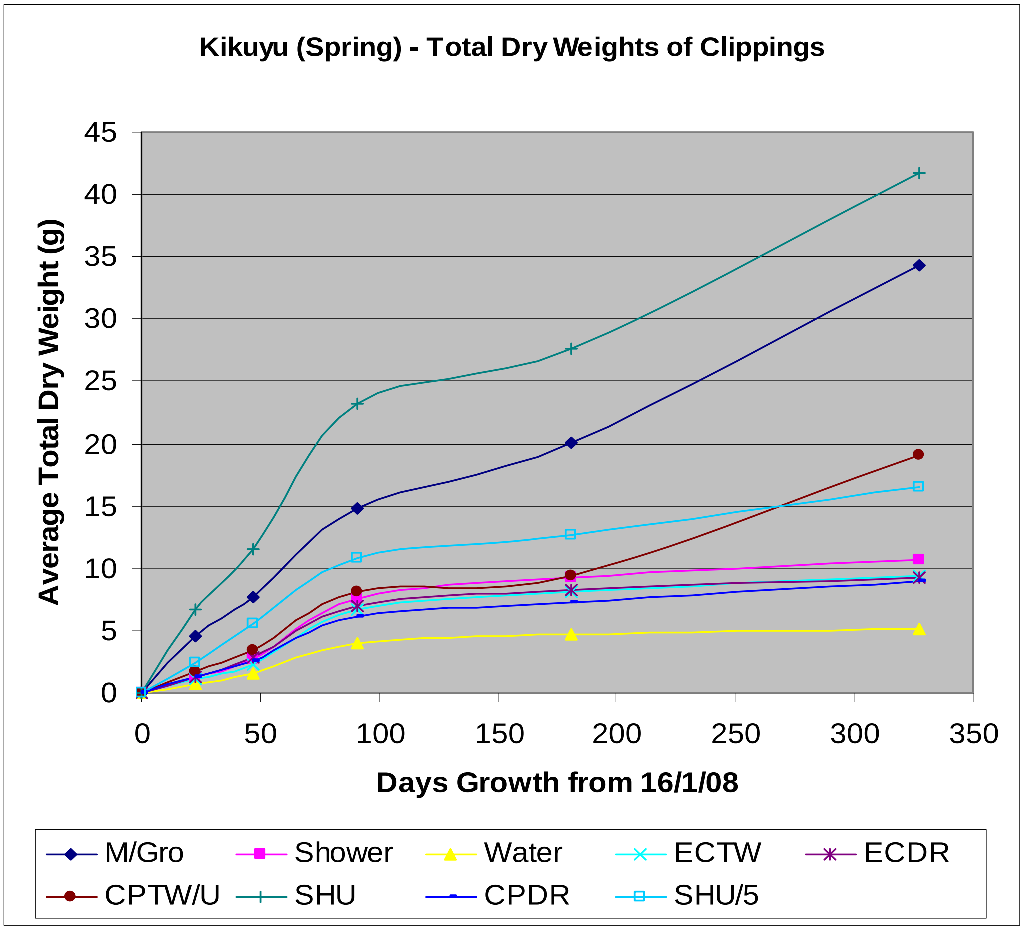

Figure 6.16 – Average Running Total Dry Weights (g) of Kikuyu (Spring) clippings collected for each Treatment over growth period 16/1/08 to 8/12/08

It appears from the Kikuyu (Spring) results that the simpler method of measuring the tallest growth heights may be useful to give acceptable results for assessing Kikuyu growth, especially when it is not feasible to determine the dry weights of clippings. The plots showing running total growth heights are similar but not identical to the plots showing the running total dry weights of clippings. The main differences in results are that the height method shows ECTW produced more growth height than Shower and CPTW, and that the total result due to CPTW and CPTWU was less than that due to SHU/5, although CPTWU was starting to catch up to SHU/5. Both graphs show Water as the least contributor to long term Kikuyu growth followed by CPDR, and ECDR, and that the treatments without added urine or plant food produce slower rates of growth, as judged by the steepness of the plots. Both graphs also show SHU as the largest contributor to growth followed by the other treatments containing added nutrients M/Gro, SHU/5, and CPTWU.

6.4.2 Growth heights of Kikuyu (Summer)

As for the Tall Fescue and the Kikuyu (Spring) samples the growth height measurements are plotted on a single graph as running total growth heights (Figure 6.17), and as a reference for comparison a graph showing the running total dry weights of Kikuyu clippings, which were obtained at the same times, is provided (Figure 6.18).

The results for the 392 days of growth from 3/1/08 to 29/1/09 are shown in Figure 6.17, and it was determined that:

- SHU produced the largest average running total growth height during the 392 days of growth, followed by CPTW SHU, and M/Gro.
- In a similar way as for Tall Fescue (Summer) the running growth heights due to M/Gro lagged behind those due to CPTW SHU, but caught up right at the end of the treatment period (see Tall Fescue results in Figure 5.28).
- The effect of urine was shown by SHU producing 168% more average total growth height than Shower, and CPTW SHU producing 121% more than CPTW Shower.
- There appears to have been a spurt in growth heights during early spring (after Day 249).

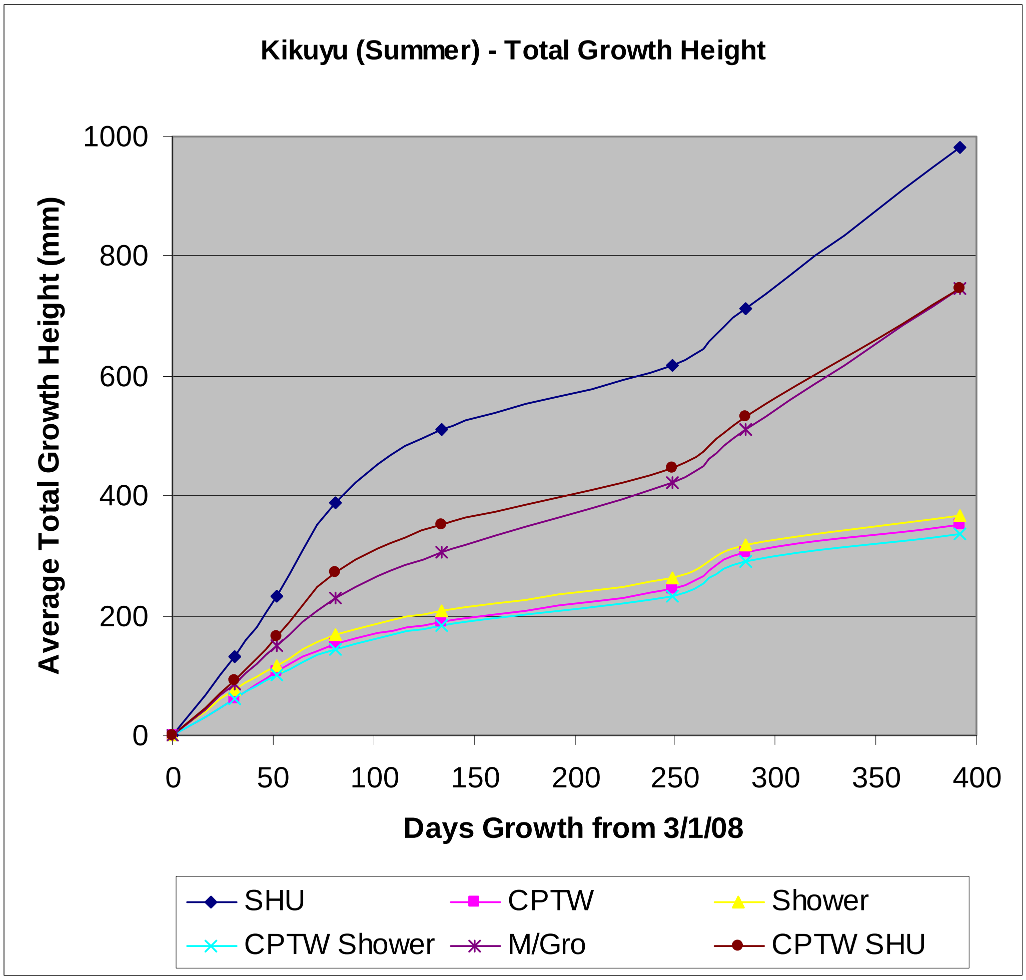

Figure 6.17 – Average Running Total Growth Heights (mm) of Kikuyu (Summer) for each Treatment, over period 3/1/08 to 29/1/09

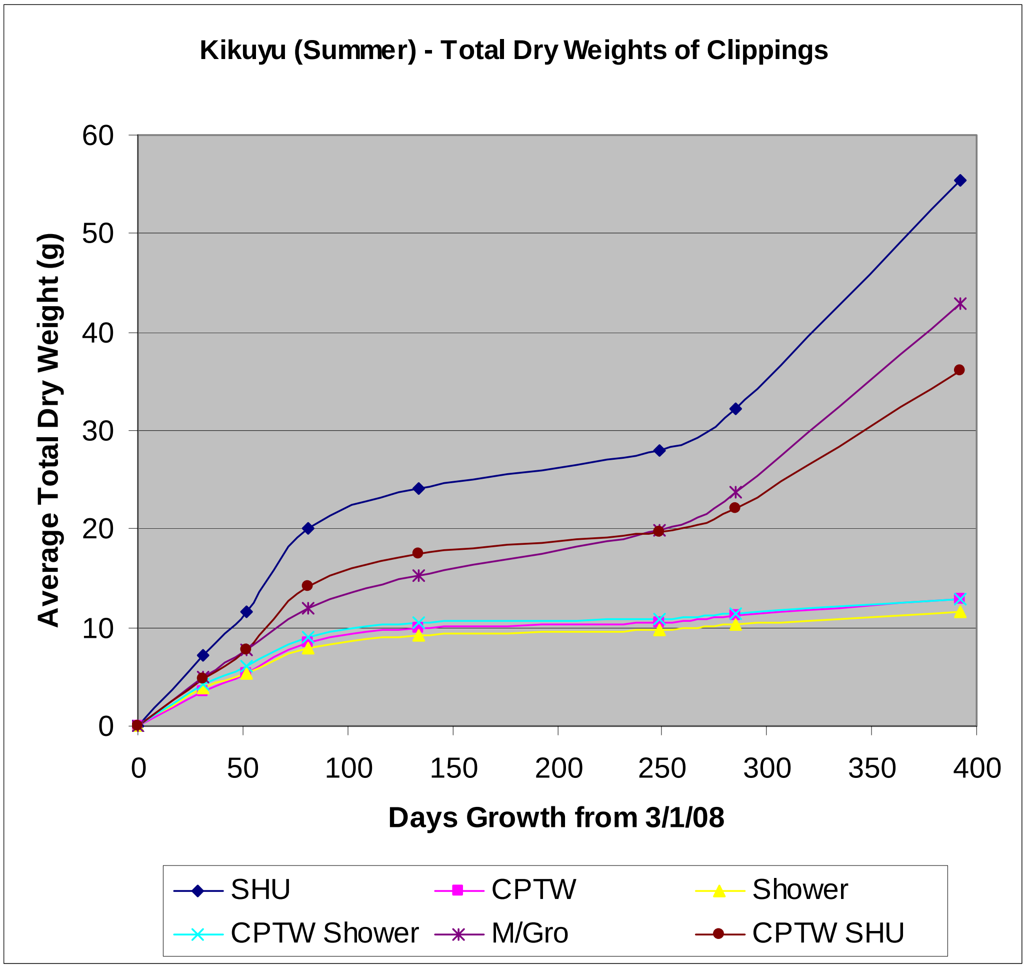

Figure 6.18 – Average Running Total Dry Weights (g) of Kikuyu (Summer) clippings collected for each Treatment over growth period 3/1/08 to 29/1/09

The Kikuyu (Summer) results confirm that the simple method of measuring the largest turf heights can be used to give acceptable results for Kikuyu growth. Both the running total growth heights and the running total dry weights graphs are similar but not identical. Both methods show that SHU was the greatest contributor to Kikuyu growth, with CPTW SHU and M/Gro producing less but very good rates of growth, especially during the warmer months. Both methods also show that the treatments which did not contain added urine or plant food i.e. Shower, CPTW Shower, and CPTW produced the least overall growth, and at slower rates as judged by the steepness of the plots.

6.5 Urine addition to poor growing Kikuyu (Spring)

The aim of the experiment was to determine how the Kikuyu (Spring) samples that were showing poor growth after eleven months of watering with the five treatments; Water, Shower, ECDR, ECTW, and CPDR would respond if 0.5% v/v urine was added to the treatments. It had already been seen that after CPTW was changed to CPTWU with the addition of 1% v/v urine from 31/7/08, the eventual result was well growing Kikuyu turf. The samples previously treated with M/Gro and CPTWU were included in the experiment for comparison, and all the treatments containing 0.5% v/v urine had '0.5U' added to their label codes for identification, including CPTWU which from 12/10/08 had the urine content reduced to 0.5% v/v.

This experiment was commenced seven days after concluding the initial experiment on the Kikuyu (Spring) samples, and was conducted on the same turf samples, which however were first subjected to soil core sampling. The experiment ran for 85 days over the Summer months from 15/12/08 to 10/3/09 and involved two cutting sessions, the first over the three days 6/2/09 to 8/2/09, and the second was completed on 10/3/09. No cutting was done on 7/2/09 when the unbearable maximum temperature of 47.3ºC was recorded.

The effect of adding 0.5% v/v urine to the five treatments was evident by the time of the first cutting session, when the Kikuyu (Spring) samples were showing increased growth. The effect was however more pronounced during the second growth period 6/2/09 to 10/3/09, in which the previously poor growing Kikuyu (Spring) samples produced increased growths that almost equalled, or were within 25.3% of the growth produced by M/Gro treatment.

The results are shown in Figure 6.19, and from the second growth period it was determined that:

- The average daily growth produced by Shower 0.5U was just 0.2% smaller than due to M/Gro.
- The average daily growth results produced by ECTW 0.5U and CPDR 0.5U were respectively only 4.6% and 5.4% smaller than produced by M/Gro.

- The smallest average daily growths were produced by Water 0.5U and ECDR 0.5U. These were respectively 24.3% and 25.3% less than produced by M/Gro.
- The largest average daily growth was produced by CPTWU 0.5U which was only 9.1% and 9.3% respectively larger than due to M/Gro and Shower 0.5U.

The differences in growth between CPTWU 0.5U treated Kikuyu and the other Kikuyu (Spring) samples receiving urine laced treatments were not as great over the three month experiment as occurred with the Tall Fescue (Spring) samples, even though the CPTWU 0.5U treated samples were subjected to urine for four months longer.

All the seven treatments M/Gro, CPTWU 0.5U, CPDR 0.5U, Shower 0.5U, Water 0.5U, ECTW 0.5U, and ECDR 0.5U produced average daily growths with differences between them that were not statistically significant ($p = 0.060$). Excluding CPTWU 0.5U, which produced the largest growth and which was subjected to longer urine treatment, raised the probability to 0.152 for the remaining six treatments. The previously poor growing Kikuyu samples were now showing statistically similar growth to that produced by M/Gro treatment.

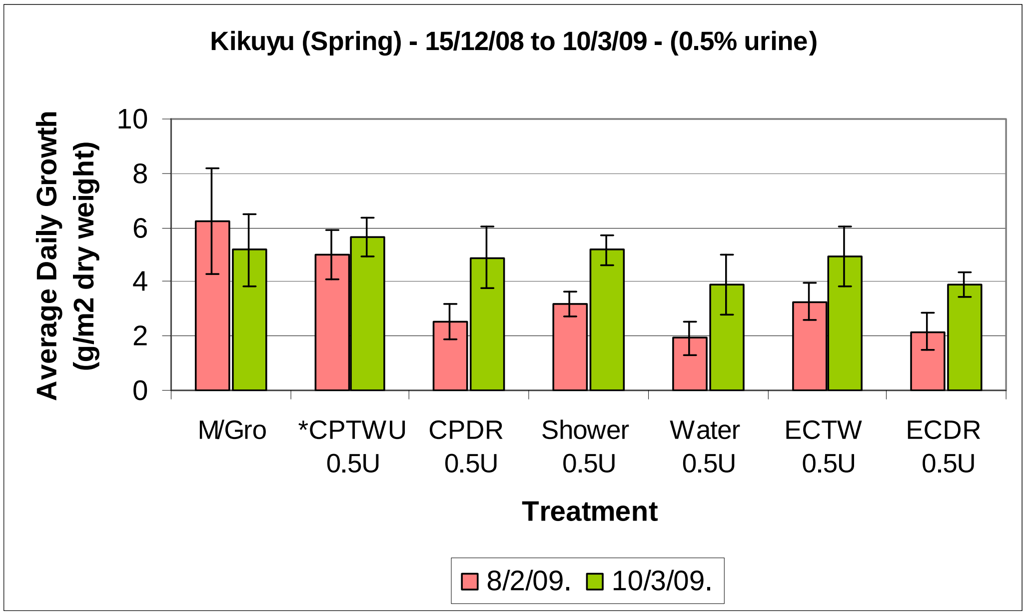

Figure 6.19 – Average Daily Growth (g/m² dry weight) of Kikuyu (Spring) over the two periods 15/12/08 to 8/2/09 and 6/2/09 to 10/3/09 (Error bars indicate one standard deviation)

6.6 Increased watering of SHU treated Kikuyu (Spring)

In general 300ml of SHU was considered to be sufficient watering during hot weather for the Kikuyu samples growing in the pots. None of the Kikuyu samples subjected to the various treatments ever looked likely to partly die off if a watering session was missed. It however was observed on several occasions during hot weather, that the fast growing Kikuyu treated with SHU could show signs of wilting about an hour before Kikuyu treated with Water or Shower.

The aim of this experiment was to determine how Kikuyu which had been subjected to 11 months of urine would grow during very hot weather, if the volume of the urine containing SHU was doubled to 600 ml for each watering session. The volume of added urine would therefore also be doubled to 6ml per watering session.

The experiment ran for 5 weeks from 25/12/08 to 31/1/09 and was conducted on the Kikuyu (Spring) samples which had received SHU treatment from 16/1/08, and had been subjected to soil core sampling before the experiment. During the eighteen days from 13/1/09 to 30/1/09 the samples successfully survived five days of maximum temperatures ranging from 34.9º C to 41.1º C plus a massive three day heatwave of maximum temperatures 43.4º C, 44.3º C, and 44.2º C.

All five replicates grew well and withstood the hot drying conditions. The results are displayed in Figure 6.20 from which it can be seen that there is greater variation between the daily growths of the five Kikuyu replicates, than was previously seen with the Tall Fescue samples (Figure 5.27) that were subjected to the same experimental conditions. The difference between the largest and smallest daily growth results over the five week period was 47.7%.

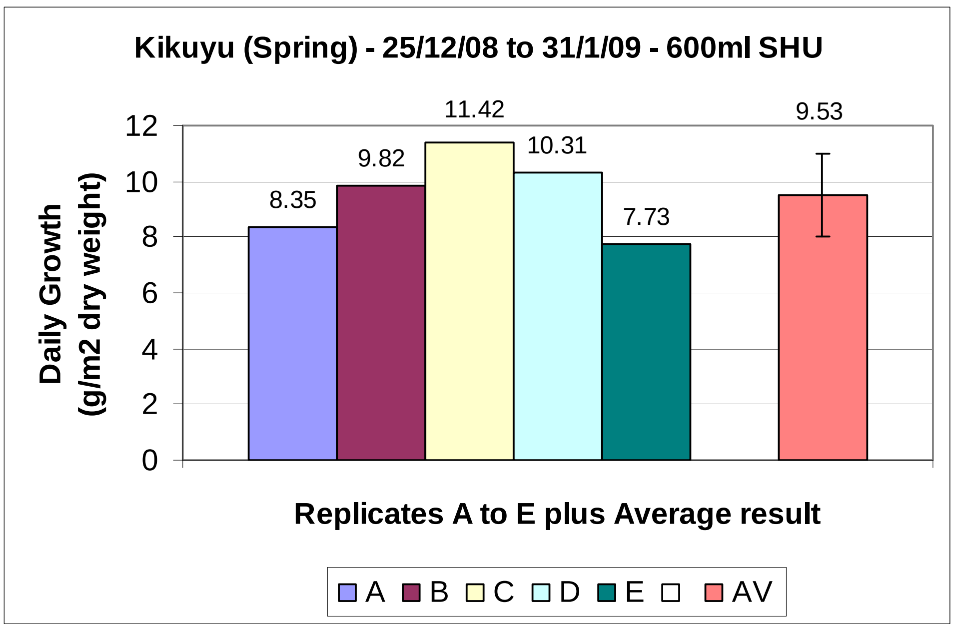

Figure 6.20 – Daily Growth (g/m² dry weight) per Pot of Kikuyu (Spring) receiving 600ml of SHU each watering session between 25/12/08 and 31/1/09 (Error bar indicates one standard deviation)

The following Chapter 7 discusses the results, outlined in Chapters 5 and 6, of the experiments in growing Tall Fescue and Kikuyu turf species with several different types of greywater, which can be sourced from a shower and a laundry.

Chapter 7: Discussions & Conclusions: Responses of Two Turf Species to Greywater

7.1 Introduction

The nature and consistency of the results reported in Chapters 5 and 6, show that untreated greywaters sourced from the bathroom or laundry are suitable for use as sources of water, for the growth of plants in gardens in south eastern Australia. Throughout the experiments that ran across the four seasons, rates of growth for both Tall Fescue ‘spring growing’ or C3 photosynthesis, and Kikuyu ‘summer growing’ or C4 photosynthesis grasses watered by specific greywaters, were at least as high, and in most cases higher than by water alone. There was also clear evidence that, where a specific greywater did not contain sufficient nutrient levels for growth of plants, the growth of each of the two types of turf was restricted. This restriction was overcome when nutrients were subsequently added.

Table 7.1 below provides a summary of the turf growth responses to the greywater treatments that were used in these experiments, as compared to the growth due to water only. Further discussion of the effects of the greywaters on the two turf species then follows.

Table 7.1 – Summary of turf growth responses to the greywater treatments – Growth rates are compared to the growth due to water only

Treatment	Response	Comments & Conclusions
Water Only	Control	Point of comparison for all other treatments
CPDR – (Cold Power® Deep Rinse) ECDR – (Earth Choice® Deep Rinse) CPTW – (Cold Power® Total Wash) ECTW – (Earth Choice® Total Wash) Shower water. CPTW Shower (50/50 blend of CPTW and Shower water).	All the treatments produced small increases in growth compared to water only, with the dilute CPDR and ECDR producing the least increase. Turf growth was poor in the longer term with all treatments. Phosphate containing CPTW did not produce significantly greater growth than non phosphate ECTW	All of the treatments can be used as a source of water on turf but produce little additional growth than water. To sustain good turf growth over a long period some form of plant nutrients needs to be added. Some of the phosphate may not have been available to the turf by being locked up in the soil, or some of the complex phosphate may not have hydrolysed to a plant usable form.
M/Gro – (Miracle-Gro® All Purpose plant food)	Controlled addition of nutrients. M/Gro produced considerably more turf growth than water and also than all the treatments without added urine.	Added as a solution in water every 14 days. Produced good Tall Fescue and Kikuyu growth over the experimental period.
SHU – (Shower water with 1% v/v urine) SHU/5 – (Shower water with 0.2% v/v urine)	Overall SHU produced significantly more growth than M/Gro treatment, and SHU/5 produced similar Tall Fescue growth to M/Gro but less Kikuyu growth. Both treatments produced considerably more growth than water and also more than the equivalent treatments without added urine.	Added every watering session. During long periods of hot dry conditions daily watering was required, with the result that the SHU treatment produced excessive growth. It is recommended that under those conditions the urine level be kept between 0.2%v/v and 0.5%v/v, especially when watering Tall Fescue turf.
CPTW SHU (50/50 blend of CPTW and SHU) contained 0.5% v/v urine.	Overall this treatment produced similar turf growth to M/Gro. Produced considerably more growth than water and also all the treatments without urine.	Added every watering session. Produced good turf growth. Urine level, for daily watering of Tall Fescue during long periods of hot drying conditions, was considered to be at maximum level.

7.2 Effect of greywaters on Tall Fescue turf

Across the four seasons of the experiments the measured growth rates of Tall Fescue produced by water were the lowest; however the results did not differ statistically significantly to the growth rates produced by the two deep rinse greywaters CPDR and ECDR. These were the only *dilute* greywaters used in the experiments, and so judging by their similar effects on growth rates compared to water, plus their low nutrient contents as shown in Chapter 3 (Figures 3.1 and 3.2), it was not surprising that laundry rinse water is often recommended for addition to gardens as a safe supplement water source, on gardening programs or in publications such as *Grey water – recycling water at home* (Better Health Channel 2011). All of the greywaters added to Tall Fescue produced rates of growth that were at least as high as the water control, and in some cases significantly higher growth resulted. For example in Chapter 5 (Figure 5.2) the urine free treatments CPTW, Shower, and ECTW produced significantly more growth than water, and the urine containing treatments SHU and SHU/5 produced even more growth. None of the greywaters produced detrimental growth effects, which further suggested that the greywaters tested, were suitable to be used untreated on Tall Fescue turf. Pinto, Maheshwari & Grewal (2009) also found that there were no apparent detrimental effects on plant growth of a very different plant type, when using greywater irrigation to grow silver beet. Similar results were also found by Misra, Patel & Baxi (2010) in growing tomatoes with laundry greywater, and by Alfiya et al. (2012) in growing Ryegrass with mainly shower and washbasin greywater.

As the experiments progressed, there was clear evidence that growth rates produced by water and the urine free treatments CPTW, CPDR, ECTW, ECDR, Shower, and CPTW Shower were limited because of a lack of nutrients. On the other hand Tall Fescue (Spring) samples treated with 0.2% v/v urine consistently produced growth rates across summer, autumn, and winter as high as those treated with Miracle-Gro® plant food, and Tall Fescue (Summer) samples treated with 0.5% v/v urine produced greater growth during summer and autumn. The poor growth rates resulting from a lack of nutrients as exhibited by the four undiluted urine free greywaters CPTW, ECTW, Shower, and CPTW Shower, were in agreement with the findings of researchers such as Ridderstolpe (2004), and Jefferson et al (2004), who considered that greywater was usually low in nutrients required by plants for growth. The limitation in turf growth because of a lack of nutrients in greywater treatments was further highlighted by the significant response in increased growth rates, after the addition of 0.5% v/v urine to several greywaters. These greywater treatments with added urine were used on poor growing turf samples, which had previously been treated for 13 months with urine free greywaters. The results indicated that if urine free greywaters were used for growing turf then some form of added plant nutrients was required to produce good strong growth.

7.2.1 Effect of urine on Tall Fescue turf

For treatments to which urine was added, growth rates were markedly higher than for water or other greywaters. Tall Fescue samples that were treated with SHU (1% urine in shower water) produced considerably more turf growth over the whole experimental period than the samples receiving all the other treatments, including M/Gro, SHU/5 (0.2% v/v urine in shower water), and CPTW SHU (0.5% v/v urine in a 50/50 blend of CPTW and shower water). The effect of urine on Tall Fescue growth was demonstrated from early in the experiment by the considerably greater growth of samples treated with SHU, and to a lesser extent with SHU/5, when compared to the growth produced by shower water alone.

As well as promoting growth the urine enhanced the green colouration of the Tall Fescue turf, with the colour depth being dependant upon the concentration of the urine in the greywater, and upon the frequency of watering. SHU/5 had the least effect on enhancing the greenness of the turf, whereas during hot months when watering occurred almost on a daily basis, the SHU treated Tall Fescue samples were so dark green coloured, that sometimes from a distance they appeared to be almost black coloured. This dark green colouration was likely to be due to nitrogen over-fertilisation (Germer, Addai & Sarpong 2009).

The nitrogen content of M/Gro was determined to be 474 mg/l – N (see Section 3.5.2), and so M/Gro solution therefore averaged 3.4 times more nitrogen than SHU, 10.9 times more than SHU/5, and 7.1 times more than CPTW SHU. M/Gro treatment (200 ml) was however applied every 14 days, (with only tap water on watering days in between), whereas a minimum of 300 ml of SHU, SHU/5, CPTW SHU, and other greywaters were used to water the plants as needed, often daily during very hot dry conditions. If the conditions required that the turf specimens be watered daily then first SHU, followed by CPTW SHU, and finally SHU/5 would introduce more nitrogen to the plants over the fortnight than M/Gro, although not in the one application.

With the SHU treated Tall Fescue samples there was an ever present risk on very hot drying days that parts of a sample could start to die off, if watering was missed or delayed. Increasing the watering volume overcame the risk, as was demonstrated by the SHU treated Tall Fescue samples surviving very hot heatwave conditions experienced in late January 2009. The extra watering volume that was needed to maintain good growth in SHU treated Tall Fescue was not in agreement with the aim of maintaining a healthy lawn with limited greywater supply. If frequent watering of Tall Fescue with greywater is required during very hot dry conditions, the use of a urine level as low as 0.2% v/v and no more than 0.5% v/v is preferable, or urine at 1% v/v should not be added to the greywater every time. During cooler months when the requirement for watering with greywater was less frequent, the use of SHU promoted good turf growth and colour, and there was never any need for increased watering volume. The effects of adding 1% v/v urine to greywater could take up to four weeks to be visibly noticeable, so there is adequate time at community centres to increase or decrease the concentration, depending upon whether greater or lesser growth is required, or depending upon the weather conditions. In domestic situations it is unlikely that each batch of shower water will contain 1% v/v urine and so the problem may not arise.

7.2.2 Effect of laundry phosphate on Tall Fescue turf

From the Tall Fescue growth results produced by the laundry greywaters CPTW and ECTW, it cannot be concluded that significantly more growth will result, if a phosphate containing laundry detergent is used rather than a phosphate free laundry detergent. During the first Tall Fescue (Spring) growth period 9/11/07 to 31/12/07 the non-phosphate based ECTW produced 23.1% higher average daily growth than the phosphate based CPTW, a result that was statistically significant ($p = 0.025$). Over the next two growth periods to 10/7/08, CPTW produced up to 38.1% more growth than ECTW however the growth differences during both periods were not statistically significant. A fourth period comparison against CPTW treatment on Tall Fescue (Summer) samples showed that ECTW produced 16.7% more growth but the difference was not statistically significant.

Perhaps the general lack of nutrients for turf growth in the urine free greywaters used may have affected the results. The average phosphorus content of CPTW at 22.6 mg/l – P was just 5% of the phosphorus content of M/Gro and the nitrogen content of 13.6 mg/l – N was just 2.9% of the nitrogen content of M/Gro (see Figures 3.1 and 3.2). Even though CPTW had more than 60 times the amount of phosphorus than ECTW, the level of nutrients in CPTW may not have been sufficient to encourage significant growth compared with ECTW. Some of the phosphorus in CPTW may not have been available to the turf specimens. Steen (1998) reported that a high proportion of phosphorus can be locked up in soil, with only 15-25% being available to plants in the first year. On the other hand, could some of the complex phosphorus compound in CPTW have passed through the pots, before it was hydrolysed to a plant usable form? It is also possible that, in the treatments with greywater derived from detergent with elevate phosphorus, the limiting factor for growth was availability of nitrogen rather than phosphorus. In all cases the addition of urine to Tall Fescue increased growth, indicating that nitrogen was a limiting factor.

7.3 Effect of greywaters on Kikuyu turf

Throughout the experiment all of the greywaters (with and without urine) that were added to Kikuyu produced rates of growth that were measured to be higher than the water control. In the early stages, even though the average growth rates produced by the urine free treatments ECTW, ECDR, Shower, CPTW, and CPDR appeared to be greater than that due to water (see Figure 6.2), the difference in growth rates was not statistically significant ($p = 0.104$). It is possible that if additional replicates of each treatment had been used, these differences might have been showed to be significant. As occurred with the Tall Fescue samples, no detrimental growth effects resulting from the use of the greywaters were observed, which suggested that all of the greywaters tested were suitable to be used untreated on Kikuyu turf.

As with the Tall Fescue it was noticeable with the progression of the experiment, that the Kikuyu samples receiving the water control or the urine free treatments CPTW, CPDR, ECTW, ECDR, Shower, and CPTW Shower appeared to be suffering to varying degrees from a lack of nutrients. It appeared that under the experimental conditions, on a bed of sandy loam soil, the treatments which did not contain urine or plant food were no longer capable of sustaining good Kikuyu growth. The poor growths were not entirely due to the tendency for Kikuyu to become dormant for a couple of months over winter, because the samples receiving Miracle-Gro®, or the urine containing treatments SHU, CPTW SHU, and SHU/5 tended to grow better and have better colouration throughout the experimental period. The good growth and colour response of Kikuyu after 1% v/v urine was added to CPTW paved the way for another experiment in which 0.5% v/v urine was added to several treatments that were not sustaining good turf growth. The significant response in growth rates, after the application of the urine laced treatments to Kikuyu samples, which had previously received urine free treatments for eleven months, was additional evidence that the Kikuyu growth was being limited by a lack of nutrients. In the same way as for the Tall Fescue samples, the Kikuyu results indicated that to produce good strong growing turf with urine free greywaters, some form of added plant nutrients was required.

7.3.1 Effect of urine on Kikuyu turf

Kikuyu required a higher concentration of urine in greywater than Tall Fescue did for the turf to produce similar growth to that resulting from Miracle-Gro® treatment. The treatment SHU which contained 1% v/v urine produced the most turf over the whole period, and initially during the warmer months, the Kikuyu specimens receiving SHU treatment had greater rates of growth than the specimens treated with Miracle-Gro® plant food (M/Gro). Over the cooler months M/Gro slightly overtook SHU, and then as new warmer weather approached both growth rates tended to equalise. The treatment CPTW SHU which contained 0.5% v/v urine, tended to produce growth results which correlated more closely with the growth produced by M/Gro, for a good proportion of the experimental period. See Section 7.2.1 paragraph 3, for an explanation of how the total amount of nitrogen added to the turf specimens, with the treatments SHU, SHU/5, and CPTW SHU, could significantly increase during long hot dry conditions.

The frequent use of SHU that occurred during hot drying months did not cause significant problems, or any evidence that a sample could die if a watering session was missed or delayed. In hot weather SHU treated Kikuyu could sometimes curl the leaves about an hour before the specimens receiving the other treatments, but that was not considered to be a problem. The main problem during the very hot months that was likely to result from the frequent application of SHU, was associated with the considerably faster growth of the Kikuyu, which would require more mowing and utilise more greywater than slower growing turf. If less growth was required then the volume of urine added to the shower water or greywater could be reduced.

The use of urine had a subtle effect on enhancing the green colouration of the Kikuyu turf. The samples appeared to become greener but the increase in greenness was not as noticeable as occurred with the Tall Fescue.

7.3.2 Effect of laundry phosphate on Kikuyu turf

Over the four growth periods from 16/1/08 to 15/7/08 the phosphate based CPTW greywater produced from 8.2% to 56.5 % more average daily growth in Kikuyu (Spring) samples than the non-phosphate based ECTW greywater. The differences in growth produced by both greywaters during all four periods were not statistically significant. From these results there is a possibility that a phosphate based laundry greywater, may produce extra growth in Kikuyu turf than a non-phosphate based greywater, but the difference in growth will probably not be statistically significant. The same explanation as outlined in Section 7.2.2 paragraph 3 applies here.

7.4 Summary

Subject to the experimental conditions under which the Tall Fescue and the Kikuyu samples were grown, the following conclusions are made:

1. All of the greywaters tested can safely be used as a source of water for both Tall Fescue and Kikuyu turf, possibly for a period of at least 12 months as was the case in conducting the experiments reported in this thesis. All the specific greywaters used produced turf growth that was at least as equal and in most cases greater than the growth produced by water only.

2. Phosphate containing laundry sourced greywater did not produce a statistically significant increase in turf growth than that produced by a non-phosphate containing laundry greywater. Perhaps the availability of phosphate may have been an issue, or possibly those plants continued to be deprived of nitrogen for full growth, so the addition of phosphorus via greywater containing elevated levels of phosphorus did not result in significantly higher growth rates.

3. With both turf varieties, all the samples that were treated with water, or with the greywaters that did not contain added urine or plant food, increasingly showed poor growth as the experiment progressed. The greywaters included shower water, and total wash and deep rinse laundry waters. There was evidence that the poor growth rates, to varying degrees, were thought to result from a lack of plant nutrients, in particular lack of nitrogen. The conclusion was that under the experimental conditions on a bed of sandy loam soil, water or any of the greywaters which did not contain added urine or plant food, were not capable of sustaining good Tall Fescue and Kikuyu growth for the whole trial period. Some form of plant nutrient was needed to be used to maintain good turf growth.

4. Human urine can be used to improve the nutrient levels of greywaters that are used to water lawns or sports ovals (subject to appropriate health protocols). The addition of urine to greywaters can achieve good turf growth and reduce the need for use of lawn fertilisers. The urine can be easily added to the

greywater such as by urinating while having a shower, or at community centres by diverting some of the urine from the urinals to the collected greywater supply. Urine addition to plain tap water should also be suitable for encouraging good turf growth, as was demonstrated in experiments towards the end (see Figures 5.24 and 6.19).

5. Both species of turf responded well when watered regularly with shower water containing urine, or a blend of laundry and shower waters also containing urine. Urine concentrations of 1% v/v, 0.5% v/v, and 0.2% v/v were used in the experiments i.e. levels that could occur if a person urinated while having a shower. The results indicate that the addition of urine to greywater can significantly increase the growth of both Tall Fescue and Kikuyu turf, especially when urine levels of 1% v/v or 0.5% v/v are used. The addition of urine also significantly increased the green colouration of the Tall Fescue and slightly improved the colour of Kikuyu.

6. Under the experimental conditions both turf varieties treated with Miracle-Gro® All Purpose plant food (M/Gro), showed good increased growth and improved colour. For a good proportion of the trial period, the growth of Tall Fescue subjected to 0.2% v/v urine was close to the growth produced by M/Gro, whereas Kikuyu tended to require a higher urine level, initially 0.5% v/v. The growth of both species of turf subjected to 1% v/v urine at each watering session was greater than the growth of turf subjected to the M/Gro treatment.

7. The urine level of 1% v/v was judged to be too high for regular application to Tall Fescue during very hot dry conditions. Under these conditions the growth of the turf was too rapid, thus requiring more watering to ensure that the Tall Fescue kept on surviving, and the turf colour sometimes was too dark green, indicating excess nitrogen addition. A urine level as low as 0.2% v/v and no more than 0.5% v/v (possibly 0.35% v/v), is recommended for frequent long term use on Tall Fescue during very hot dry conditions. During the months of moderate or cold weather conditions the use of 1% v/v urine was satisfactory.

8. The Kikuyu tolerated all the urine levels used throughout the whole experimental period, and there was never any danger of losing a sample during very hot dry conditions. The fast growth resulting from the urine level of 1% v/v could be a problem if more frequent mowing or possibly increased greywater usage, to maintain the growth, are seen as problems.

Chapter 8: Greywater Experiments on Two Types of Australian Native Flowers – Experimental Methods

8.1 Introduction

More than a decade of drought conditions in south-eastern Australia between 1997 and 2009, and associated water restrictions had resulted in an emerging trend for sustainable drought resistant landscaping for which some Australian native plants were proposed to be suited (Department of Environment and Natural Resources 2010; Sustainable Gardening Australia 2011). Australian native plants were suggested as being good water wise plants because, once established, they required little water, very little maintenance, and low nutrients (Hahn 2008; Sustainable Gardening Australia et al 2006).

The two types of native flowers selected for these experiments were (1) a perennial flowering plant and (2) a tuberous rooted plant. Each of these species is native to the open grasslands of the Basalt Plains of Victoria. The two native plant species used were Scaly Buttons *(Leptorhynchos squamatus)* and Small Vanilla Lilies *(Arthropodium minus)*, both of which were purchased from the Iramoo Native Plant Nursery at Victoria University St Albans campus. The plants were obtained as individual seedlings in small tapered pots and the seedlings were replanted, four plants to a pot, into larger 250 mm x 250 mm square pots. Having been grown from wild seed, the plants were not uniform, and so the aim was to treat the contents of each pot as a single sample, in an attempt to reduce the effects resulting from the natural variability between seed grown plants. For further information about Scaly Buttons and Small Vanilla Lilies please refer to Chapter 2 (Sections 2.12.1 and 2.12.2).

Greywater could potentially be used to water native plants in garden settings but there were questions as to whether the nutrients or other additives in some types of greywater might negatively affect their growth. There was a lack of published information about how Australian native plants respond to the several different types of greywaters which can be sourced from a laundry or a shower facility. The studies reported below were designed to investigate this gap in our knowledge.

8.2 Pots, soil, and planting of the native flowers

8.2.1 Pots

The pots for these experiments were constructed from the same type of 12 litre square buckets as were used for the turf experiments (See Chapter 4 Section 4.2.1). The buckets were sold by Bunnings Warehouse stores and the 250 mm x 250 mm open top dimensions were assessed as being large enough to accommodate four plants.

8.2.2 Soil

The soil used for growing the native flowers was different to the soil used for growing the turf samples. Sandy loam was often used to grow turf, but native plants would likely be grown in a garden area containing different soil. The soil for growing the native flowers was an equal volume blend of three ingredients:

1. Sandy loam top soil.
2. Sieved Soil, mainly basaltic clay, obtained from the grasslands near Iramoo Native Plant Nursery.
3. Sieved partly composted Eucalyptus mulch.

The two sieved ingredients were the fines collected after passing through a plastic garden sieve with approximately 6 mm x 6 mm openings. The aim was to produce a freely draining potting soil suitable for native plants. There was a possibility that the Eucalyptus mulch added to the soil was insufficiently composted and could negatively affect the amount of available nitrogen for the plants (Doring et al. 2005; Herms et al. 2001; Wilkinson & Biala 2001). This was not considered to be a significant problem for the experiment, because the mulch used was recommended by nursery suppliers as useful in community gardens for applying to soil, as a means of conserving soil moisture. The use of mulches to conserve water was encouraged by a $30 rebate off the water bill (e.g. from City West Water).

The soil blend was placed into the pots and packed down by lightly tapping with a brick. The soil filled pots were then placed into a tub of water until it was observed that the soil was thoroughly wet. The pots were then removed to allow excess water to drain out. Two of the one hundred pots in which the flowers were grown waterlogged after heavy rain so all hundred pots were then treated with Hortico® Soil Wetter Granules.

8.2.3 Planting the native flowers

The seedlings together with attached potting mix plugs were removed from their seedling pots by gently squeezing each pot to loosen the contents, and then holding two fingers across the opening and giving each pot a sharp shake while holding it upside down. These were then planted into suitable sized holes cored into the soil in the 250 mm x 250 mm pots. A satisfactory corer was fashioned from a tall slender empty asparagus can, as this had exactly the right dimensions (note: soil corers can also be readily purchased for this purpose). Four seedlings were planted into each 250 mm x 250 mm pot as explained previously.

8.2.4 Initial care of the planted native flowers

The flowers were planted into pots at the start of a hot summer in early December 2007. There was a considerable amount of exposed soil area around the four plants which made the soil susceptible to evaporation and weed growth. Sugar cane mulch was therefore carefully placed around the plants and under every stem that was lying on the soil. Seasol® seaweed concentrate was then added to the pots, and the plants were also fed once with Miracle-Gro® all purpose plant food. Tap water was applied to the native flowers over the summer but no significant growth occurred until the autumn. In fact the Small Vanilla Lilies died back and were cut down, and all dead stems of the Scaly Buttons were either cut off or broken off by the wind. This is a normal part of the Scaly Buttons yearly growth cycle. The addition of experimental greywaters was not commenced until new growth was evident, with the Scaly Buttons receiving their first greywater treatments on April 9, 2008, and the Small Vanilla Lilies on May 22, 2008.

8.3 Watering of the native flowers

8.3.1 Rain and watering treatments

Just as in the case of the turf samples, naturally falling rain was considered to be part of the experimental watering program for the native flowers. The measured quantity of monthly rain that fell during the experimental period can be found in Appendix A. Water treatments that were applied to the native flowers included Miracle-Gro® solution, tap water, and several greywaters. The standard watering quantity per pot was 300 ml and this was applied as deemed necessary by judging the dampness of the soil under the sugar cane mulch, and taking in factors such as drying conditions, rain, and ambient temperatures. The 300 ml was added to each pot from a sprinkling cup in the same way that was used to water the turf samples. The growth started as winter was approaching and ended in the summer, so the watering frequency varied from daily to several days apart. Miracle-Gro® solution was added in exactly the same way that it was added to the turf samples, and the minimum treatment interval was 14 days as for the turf samples.

8.3.2 Watering treatments used on the native flowers

1. **Water** – Melbourne tap water.
2. **M/Gro** – Miracle Gro® solution
3. **CPTW** – Cold Power Total Wash.
4. **CPDR** – Cold Power Deep Rinse.
5. **ECTW** – Earth Choice Total Wash.
6. **ECDR** – Earth Choice Deep Rinse.
7. **Shower** – Shower water.
8. **SHU/2** – Shower water + 0.5% v/v urine.
9. **SHU/5** – Shower water + 0.2% v/v urine.
10. **CPTW Shower (or CPTW & Shower)** – Equal blend of CPTW and Shower.

For a detailed description of the above watering treatments please refer to Section 3.5 and to Sections 3.5.1 and 3.5.2 for the Total P and Total N contents of the treatments.

8.4 Layout of pots containing the native flowers

The hundred pots containing the Scaly Buttons and Small Vanilla Lilies were grouped into five replicate sets. Each replicate set labelled L, M, N, P, or Q contained ten pots of Scaly Buttons and ten pots of Small Vanilla Lilies. Ninety two of the pots containing the native flowers were positioned to the East (left) side of the turf samples, with the remaining eight pots being placed in front (North side) of the turf samples, within the overall experimental layout (see Chapter 4). The L set of pots had to be arranged in a 2 x 10 configuration instead of 4 x 5 to ensure that all pots were away from the canopy of a tree. The Q set was split into 2 parts to ensure that all the pots were clear of likely winter shade from a nearby house and associated shrubs.

An online random number generator was used to select the pots for each replicate set (StatTrek 2008). The same generator was also used to select the pattern of treatments to be applied to the samples of only one set (M). For the same reasons as with the turf samples, such as reducing potential watering errors, the patterns of treatments to be applied to the other replicate sets were alternately interchanged along the centre line of experimental pots.

In Chapter 4 the photograph (Figure 4.1) shows the actual layout of the pots containing the native flowers and the turf samples. A diagrammatic illustration of the layout of the pots containing the two types of native flowers, the patterns of the treatments applied, and the set and pot numbers is shown in the following Table 8.1.

Table 8.1 - Layout of Pots for Scaly Buttons & Small Vanilla Lilies Experiments

			SB	**SVL**						
			L-Set							
		L1	SHU/5	SHU/5	L2					
		L3	ECDR	ECDR	L4	Kikuyu and Tall Fescue Pots				
		L5	Water	Water	L6					
		L7	Shower	Shower	L8					
		L9	ECTW	ECTW	L10					
		L11	CPTW	CPTW	L12					
		L13	CPDR	CPDR	L14					
		L15	M/Gro	M/Gro	L16					
		L17	CPT Sh	CPT Sh	L18					
		L19	SHU/2	SHU/2	L20					
	SB	**SVL**	**SB**	**SVL**						
	M-Set									
M1	ECDR	ECDR	SHU/5	SHU/5	M4					
M5	Shower	Shower	Water	Water	M8		**S**			
M9	CPTW	CPTW	ECTW	ECTW	M12		**E W**			
M13	M/Gro	M/Gro	CPDR	CPDR	M16		**N**			
M17	SHU/2	SHU/2	CPT Sh	CPT Sh	M20					
	N-Set									
N1	SHU/5	SHU/5	ECDR	ECDR	N4					
N5	Water	Water	Shower	Shower	N8					
N9	ECTW	ECTW	CPTW	CPTW	N12					
N13	CPDR	CPDR	M/Gro	M/Gro	N16					
N17	CPT Sh	CPT Sh	SHU/2	SHU/2	N20					
	P-Set									
P1	ECDR	ECDR	SHU/5	SHU/5	P4					
P5	Shower	Shower	Water	Water	P8					
P9	CPTW	CPTW	ECTW	ECTW	P12					
P13	M/Gro	M/Gro	CPDR	CPDR	P16					
P17	SHU/2	SHU/2	CPT Sh	CPT Sh	P20					
						SB	**SVL**	**SB**	**SVL**	
	Q-Set (Part 1)					**Q-Set (Part 2)**				
Q1	SHU/5	SHU/5	ECDR	ECDR	Q4 Q13	CPDR	CPDR	M/Gro	M/Gro	Q16
Q5	Water	Water	Shower	Shower	Q8 Q17	CPT Sh	CPT Sh	SHU/2	SHU/2	Q20
Q9	ECTW	ECTW	CPTW	CPTW	Q12					
	SB	**SVL**	**SB**	**SVL**						
	CODES:									
	SB = Scaly Buttons				CPT Sh = CPTW Shower					
	SVL = Small Vanilla Lilies				L1, P4, Q20 etc = Pot identification numbers					

8.5 Pests on the Native Flowers

8.5.1 Snails

With the drought conditions the general lawn area around where the experiment was set up was basically dead except for around the pots of turf and native flowers. Early in the experiment it was noticed that the foliage of some of the Small Vanilla Lilies in a couple of the pots had been partly eaten. A search around the outside of the pots with eaten flowers located a large snail. Snail bait was therefore applied to all the pots with native flowers and on the ground around the pots. This seemed to solve the problem.

8.6 Measurement of growth of the native flowers

The main methods of determining the growth of the Scaly Buttons and the Small Vanilla Lilies were carried out at the end of the growth period, using both non destructive and destructive procedures. The methods used after end of treatment harvesting included counting the flower heads, determining the dry weights of the foliage and roots, and measuring stem lengths. During the growth period there were also measurements taken on several occasions of the longest leaves or stems. Determining dry weights, counting of leaves or fruits, and measuring stem lengths are commonly reported methods of assessing plant growth (Lunt & Morgan 1999; Misra, Patel & Baxi 2010; Pinto, Maheshwari & Grewal 2009; Salukazana et al. 2006; Wood & Roper 2000).

8.6.1 Number of flower heads

This method can be used without destroying the plants. However, for the experiments reported here it was found to be easier to count the flowers after the plants had been harvested. Counting the flowers on some of the largest unharvested plants, such as Scaly Buttons treated with M/Gro or SHU/2, could be difficult because of the crowding of the four plants in each pot. Most of the plants in most of the pots were not overlapping for the duration of the experiment. The flower counts included any unopened flower heads for the Scaly Buttons, and any seed pods for the Small Vanilla Lilies.

8.6.2 Dry weights of the foliage

The Small Vanilla Lilies were harvested after 23 weeks of growth and treatment and the Scaly Buttons after 36 weeks. After the flower heads had been counted and the stem lengths measured, the foliage was cut into suitable lengths to fit into brown paper lunch bags. These were then hung up in a sheltered place to air dry for a few weeks before being placed into a laboratory oven at 70°C for 24 hours. The dry weights of the foliage were determined by first weighing the bags containing the dry foliage, and after that weighing the empty bags.

8.6.3 Dry weights of the roots

The roots were carefully removed and washed to remove adhering soil and after the excess water dried off, the roots were placed into brown paper lunch bags. The roots were then air dried and oven dried, and had the dry weights determined in the same way as the foliage.

Removal of the roots differed between the two types of plants. The Small Vanilla Lilies had small tuberous roots which could be dug out while still attached to the foliage. A mark was painted on the stems to indicate ground level before digging the plants out of the soil, as a reference for later cutting. The Scaly Buttons presented a problem because their roots were very long and fibrous and often intertwined within the pots, and so they could not be dug out without cutting them up. The problem was discovered on digging out the plants from the first pot i.e. Q15 which was treated with Miracle-Gro ® solution. It was found that a considerable amount of roots had remained in the soil, and so the roots from pot Q15 had to be excluded from calculating the average dry weight and the standard deviation.

The adopted procedure for the Scaly Buttons was to first cut the foliage at ground level, and then to remove the large soil plugs containing the roots from the pots. The soil plugs were then gently broken up by hand to free up the roots.

8.6.4 Lengths of the longest stems or leaves of each plant

These measurements were taken on three occasions during the growth period and at harvest of the plants. Up to five leaves or stems were measured on each plant, but for judging the growth rates it proved to be sufficient just to use the length measurement of the one longest stem or leaf from each plant in a pot, and then to total up the four measurements from each of the four plants to produce a Length Sum for each pot. The lengths of the Scaly Buttons stems were measured only to the end of the sections covered in leaflets. The very thin bare stems which held the flower heads were not included in the measurements. The Small Vanilla Lilies had rosettes of leaves for most of the growth period until near maturity, when tall flowering stems quickly sprung up from early September.

In the following Chapter 9 the growth results of the Scaly Buttons and the Small Vanilla Lilies produced by the ten watering treatments are examined by counting the flower heads, determining the dry weights of the foliage and of the roots, and by measuring the lengths of the stems. The data is also examined statistically.

Chapter 9: Results of using Greywaters on Scaly Buttons and Small Vanilla Lilies

9.1 Introduction

The growth results for the Scaly Buttons and for the Small Vanilla Lilies are presented below in graphical form as Figures to make it easier to quickly see the growth differences, however if required the corresponding numerical data in Table form can be obtained from the author.

Plants of each of the two species were watered with the ten treatments outlined in Chapter 8 (Section 8.3.2) plus the occasional rain. As for the turf experiments, two of the treatments Miracle-Gro® solution (M/Gro) and Water were not greywaters but provided nutrient and non-nutrient based controls for comparison against the greywaters used. The main method of assessing the growth was carried out at the end of the growth period when the plants were harvested, and the number of flower heads and dry weights of the foliage and roots were determined. Measurements of the longest stems or leaves were also taken during the growth period and at the end when the plants were harvested. Details of the methods used to assess growth are outlined in Chapter 8 (Section 8.6).

The four plants in each pot were treated as a single sample in an effort to minimise the effects due to the natural variability between plants grown from seeds. For example the counts of flower heads therefore represent the sum totals for each pot and not for each plant. The averages and standard deviations were then calculated using the five totals obtained from the five replicate pots, for each treatment, rather than from the totals obtained from the twenty plants individually.

To distinguish between the four plants in a pot extra identification codes based on the compass points NW, NE, SE, and SW was used. For example the Scaly Button plant in the North West corner of pot L15 was referred to as L15-NW. The results were recorded for each plant in a pot and then summed to provide the total for the pot. This was done in case at a later date, it was determined that the results for individual plants were needed.

9.1.1 Analysis of results

The same overall methods of analysing the growth results for the Scaly Buttons and the Small Vanilla Lilies were used as for the Tall Fescue and the Kikuyu experiments, bearing in mind that the methods of collecting this growth data varied between turf grass and native plants, and so direct comparisons of growth rates between the types of plants could not be made. The Average or Mean growth results, and the Standard Deviations as presented in the Figures were calculated by using Microsoft Office Excel 2003. One-way ANOVA tests and the occasional Student's t-Tests were produced with online calculators available from *The College of Saint Benedict and Saint John's University*. The CSBSJU calculators expressed the result of each statistical calculation as a probability, assuming the null hypothesis, that there was no difference produced by the treatments. A probability (p) greater than 0.05 was taken to signify that there was no statistically significant difference produced by the treatments, while a probability less than 0.05 signified that there was a statistically significant difference.

9.2 Growth Results for Scaly Buttons

The effects of the various treatments applied to the Scaly Buttons on growth were examined by:

- Counting the number of flower heads.
- Determining the dry weights of the foliage.
- Determining the dry weights of the roots.
- Measuring the longest stem of each plant in a pot.

9.2.1 Number of flower heads

As the plants grew and produced flower heads it was obvious from a casual inspection which of the plants were being treated with M/Gro, SHU/2, and to a lesser extent SHU/5. The results are displayed in Figure 9.1 below, and the following observations were made:

- The seven treatments without added urine or plant food i.e. Water, ECDR, Shower, ECTW, CPTW, CPDR, and CPTW Shower, averaged between 29 (ECDR) and 76 (CPTW) flower heads per pot. Combined statistical comparison of all seven results showed no significant difference ($p = 0.214$).
- M/Gro produced an average of 595 flower heads per pot, followed by SHU/2 (343) and SHU/5 (162). Difference between the three results was statistically significant ($p <0.001$).
- Water and M/Gro results indicate that on average the use of Miracle-Gro® all purpose plant food increased the number of flower heads per pot by 926%.
- Results of Shower, SHU/2, and SHU/5 treatments indicate that the addition of 0.5% v/v urine to shower water (SHU/2) increased the number of flower heads per pot by 472%, and addition of 0.2% v/v urine (SHU/5) by 170%.
- Total wash CPTW averaged 77% more flower heads than deep rinse CPDR ($p = 0.093$), whereas total wash ECTW averaged 69% more than deep rinse ECDR ($p = 0.200$).
- Phosphate containing CPTW averaged 55% more flower heads than phosphate free ECTW ($p = 0.169$).

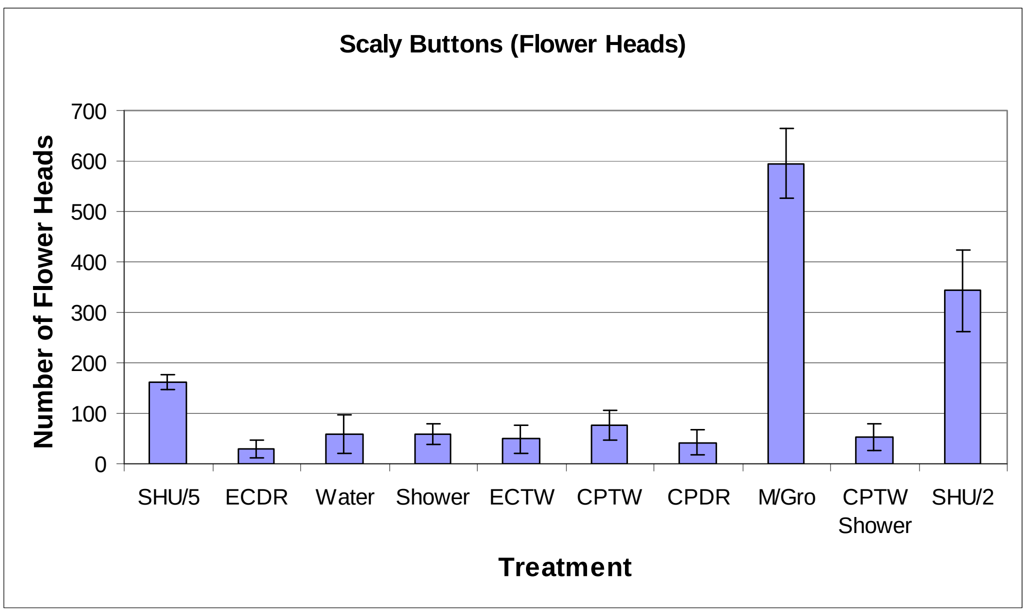

Figure 9.1 – Scaly Buttons (Flower Heads) – Average number of Flower Heads per Pot per Treatment (Error bars indicate one Standard Deviation)

9.2.2 Dry weights of the foliage

The pattern of results for the dry weights of the foliage is very similar to that obtained for the number of flower heads, which can be seen by comparing Figure 9.2 with Figure 9.1. The results for dry foliage weight for each treatment are shown in Figure 9.2, and show the following:

- The average dry weights of foliage produced by the seven treatments Water, ECDR, Shower, ECTW, CPTW, CPDR, and CPTW Shower, ranged from 4.6g (ECDR) to 7.5g (CPTW), however the difference between all seven results was not statistically significant ($p = 0.537$).
- M/Gro treatment gave the highest average foliage dry weight per pot of 55.2g, followed by SHU/2 (29.3g), and SHU/5 (15.4g). Difference between the three results was statistically significant ($p < 0.001$).
- M/Gro and Water treatment results show that on average the use of the plant food increased the foliage dry weight per pot by 736%.
- SHU/2 produced 397% more foliage dry weight than Shower water, and SHU/5 produced 161% more. Both results statistically significant ($p < 0.001$).
- Total wash CPTW averaged 46% more foliage dry weight than deep rinse CPDR ($p = 0.183$), whereas total wash ECTW produced 36% more than deep rinse ECDR ($p = 0.188$).
- Phosphate containing CPTW averaged 18.6% more foliage dry weight than phosphate free ECTW ($p = 0.464$).

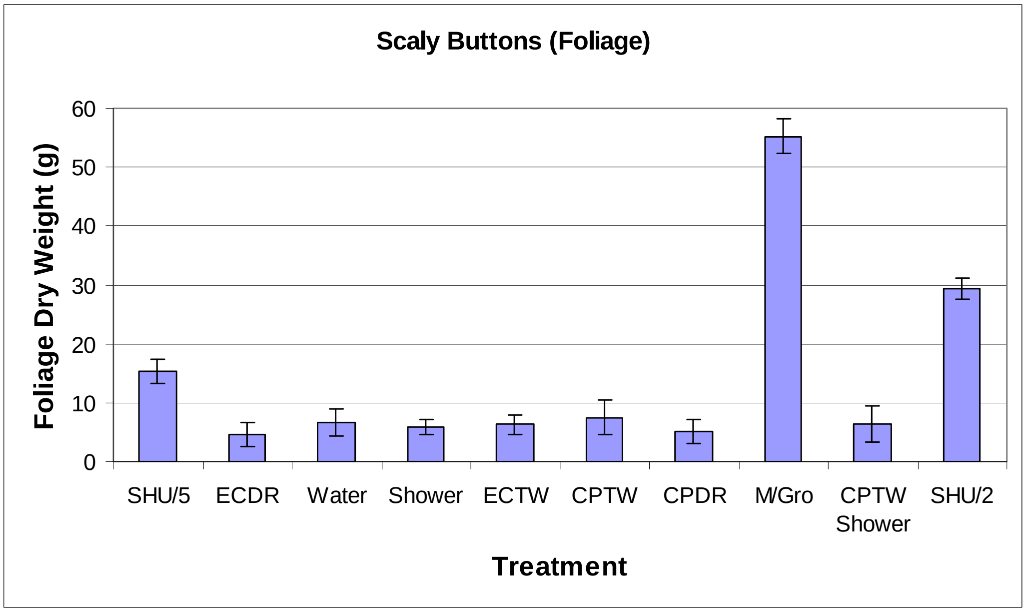

Figure 9.2 – Scaly Buttons (Foliage) **–** Average Dry Weights (g) of Foliage per Pot per Treatment (Error bars indicate one Standard Deviation)

9.2.3 Dry weights of roots

The results obtained by weighing the dry roots are shown in Figure 9.3, and the following observations were made:

- The average dry weights of roots produced by the eight treatments Water, ECDR, Shower, ECTW, CPTW, CPDR, CPTW Shower, and the urine containing SHU/5, ranged from 5.4g (CPTW Shower) to 8.2g (Water), however the difference between all eight results was not statistically significant ($p = 0.087$).
- M/Gro was still the standout treatment producing roots with an average dry weight per pot of 20.2g, which was more than twice that of the next heaviest i.e. SHU/2 with 9.6g.
- The differences between SHU/2 and the remaining treatments were considerably less for root weights than for the number of flower heads, and foliage weights.
- Results due to M/Gro and Water treatments indicate that on average the use of the plant food increased the dry root weight by 145%.

- The addition of 0.5% v/v urine to shower water increased the average dry root weight by almost 24%, but addition of 0.2% v/v urine resulted in less than 1% increase over that produced by Shower. Difference in results between the three treatments was not statistically significant (p = 0.175).
- Total wash CPTW averaged 19% more dry root weight than deep rinse CPDR (p = 0.402), whereas ECTW averaged 30% more than ECDR (p = 0.112).
- Phosphate free ECTW averaged 13.9% more dry root weight than phosphate containing CPTW (p = 0.401).

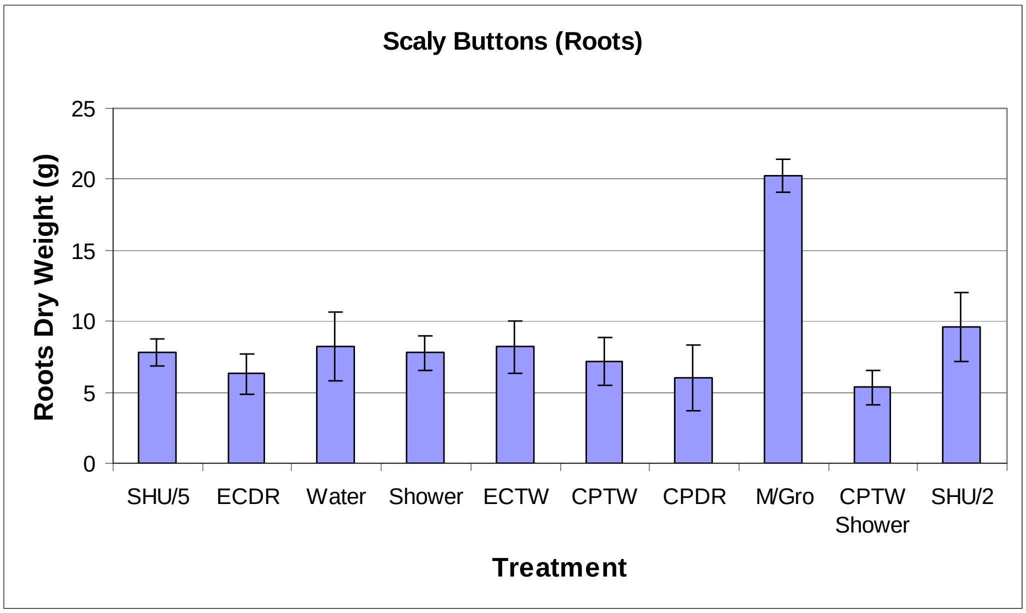

Figure 9.3 – Scaly Buttons (Roots) **–** Average Dry Weights (g) of Roots per Pot per Treatment (Error bars indicate one Standard Deviation)

9.2.4 Stem Length Sum (SLS)

This method involved measuring the longest stem from each of the four plants in a pot and adding the four measurements together to obtain the Stem Length Sum (SLS) per pot. The SLS results are shown in Figure 9.4, and the following observations were made:

- The average SLS results produced by the seven treatments Water, ECDR, Shower, ECTW, CPTW, CPDR, and CPTW Shower, ranged from 463 mm

(ECDR) to 568 mm (ECTW), however the difference between all seven results was not statistically significant (p = 0.554).

- The lowest results were due to ECDR (463mm) and Water (492mm).
- M/Gro treatment produced the largest average SLS per pot of 948mm, followed by SHU/2 (753mm) and SHU/5 (680mm). Difference between the three results was statistically significant (P < 0.001).
- M/Gro and Water results indicate an average increase in SLS of 93% due to the addition of plant food.
- The results for the three treatments SHU/2, SHU/5, and Shower, indicate a 43% average increase in SLS due to adding 0.5% v/v urine (p = 0.001), and a 29% increase due to adding 0.2% v/v urine (p = 0.021).
- SHU/2 averaged 10.7% more SLS than SHU/5 (p = 0.085)
- Total wash CPTW averaged 10% more SLS than deep rinse CPDR (p = 0.454), whereas ECTW averaged 23% more than ECDR (p = 0.155).
- Phosphate free ECTW averaged 1.8% more SLS than phosphate containing CPTW (p = 0.876).
- The SLS results do not show as great differences between the highest and lowest results as shown by the foliage dry weights.

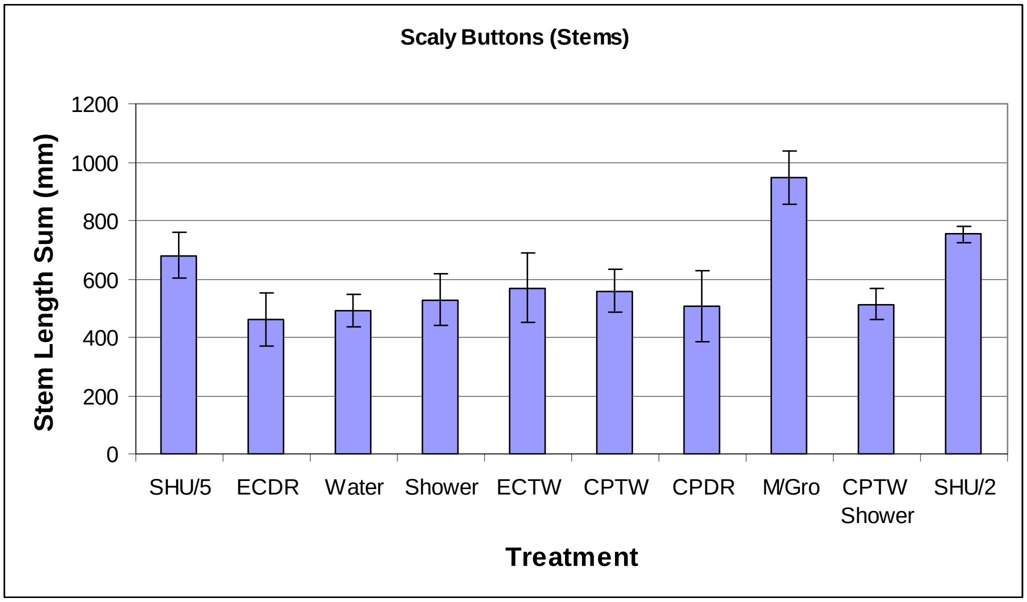

Figure 9.4 – Scaly Buttons (Stems) **–** Average Stem Length Sums (mm) per Pot per Treatment (Error bars indicate one Standard Deviation)

9.2.5 Photographs of two harvested Scaly Buttons

The following photographs (Figure 9.5) and (Figure 9.6) of Scaly Buttons removed from a SHU/2 treated pot L19, and a Shower treated pot L7 show the differences in flower heads, foliage growth, stem lengths, and roots due to the addition of 0.5% v/v urine to shower water (SHU/2). The roots are positioned as if still attached

Figure 9.5 – Scaly Buttons (Harvested) grown with SHU/2 treatment

Figure 9.6 – Scaly Buttons (Harvested) grown with Shower treatment

9.2.6 Growth measurements during the growing period

The Stem Length Sum (SLS) for each pot was determined on three occasions during the growth period, and when the plants were harvested. The harvest results have already been discussed in Section 9.2.4. The Average SLS results for the four measuring occasions are shown in Figure 9.7.

Application of the treatment solutions began on April 9, 2008 and within four weeks the M/Gro and SHU/2 treated samples showed increased average stem growth, whereas SHU/5 had similar stem growth to the total wash waters CPTW and ECTW. At twelve weeks SHU/5 produced more average stem growth than CPTW and ECTW. Overall to harvest time M/Gro produced the longest stem growth, followed by SHU/2 and then SHU/5, and of the seven treatments which did not contain added urine or plant food, the two total wash waters CPTW and ECTW produced slightly greater stem lengths than the other five treatments.

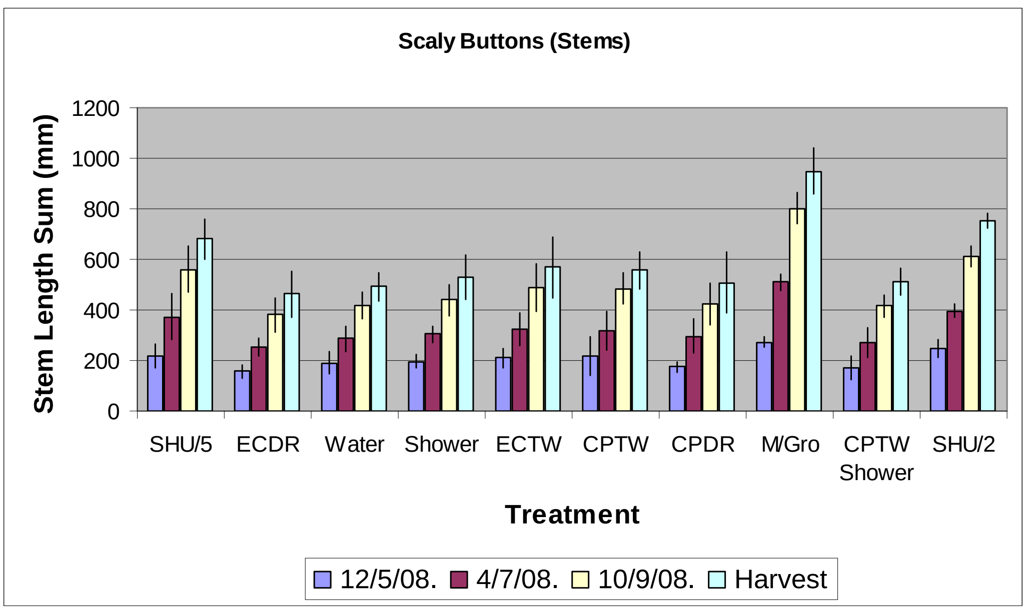

Figure 9.7 – Scaly Buttons (Stems) – Average Stem Length Sums (mm) per Pot per Treatment as determined on four occasions (Error bars indicate one Standard Deviation)

9.2.7 Early photographs of two Scaly Buttons

The following photographs (Figure 9.8 and Figure 9.9) of Scaly Buttons in a M/Gro treated pot P13 and a Water treated pot P7 show a typical difference in stem growth that had resulted from applying Miracle-Gro® all purpose plant food (M/Gro) for almost 12 weeks.

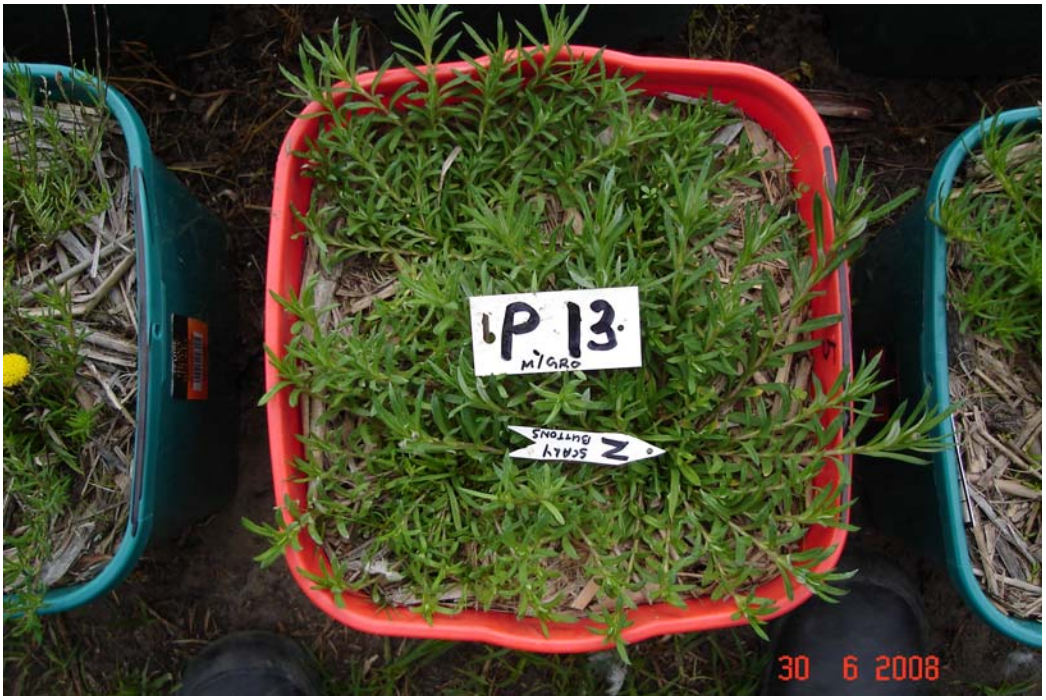

Figure 9.8 – Scaly Buttons grown with M/Gro treatment (Early growth)

Figure 9.9 – Scaly Buttons grown with Water treatment (Early growth)

9.3 Growth Results for Small Vanilla Lilies

The growth results for the Small Vanilla Lilies were examined by similar four methods as used for the Scaly Buttons i.e.

- Counting the number of flower heads and seed pods.
- Determining the dry weights of the foliage.
- Determining the dry weights of the roots.
- Measuring the longest stem of each plant in a pot.

9.3.1 Number of flower heads and seed pods

The count of flower heads and seed pods included pods which had already formed when the count was being done. The results are shown in Figure 9.10, and the following observations were made:

- The average number per pot of flower heads and pods produced by the seven treatments without added urine or plant food i.e. Water, ECDR, Shower, ECTW, CPTW, CPDR, and CPTW Shower, ranged from 188 (ECDR) to 232 (Shower), however the difference between all seven results was not statistically significant ($p = 0.548$).
- M/Gro treatment produced the largest number of flower heads and seed pods with an average of 855 per pot, followed by SHU/2 (454) and SHU/5 (338). Difference between the three results was statistically significant ($p = 0.005$).
- Water and M/Gro treatment results indicated that the use of the plant food increased the average number of flower heads and pods per pot by 338%.
- SHU/2 produced 96% more flower heads and pods than Shower ($p = 0.021$), while SHU/5 produced 46% more than Shower ($p = 0.155$).
- Total wash CPTW averaged 24% more flower heads and pods per pot than deep rinse CPDR ($p = 0.060$), whereas ECTW averaged 21% more than ECDR ($p = 0.458$).
- Phosphate containing CPTW averaged 10.1% more flower heads and pods per pot than phosphate free ECTW ($p = 0.654$).

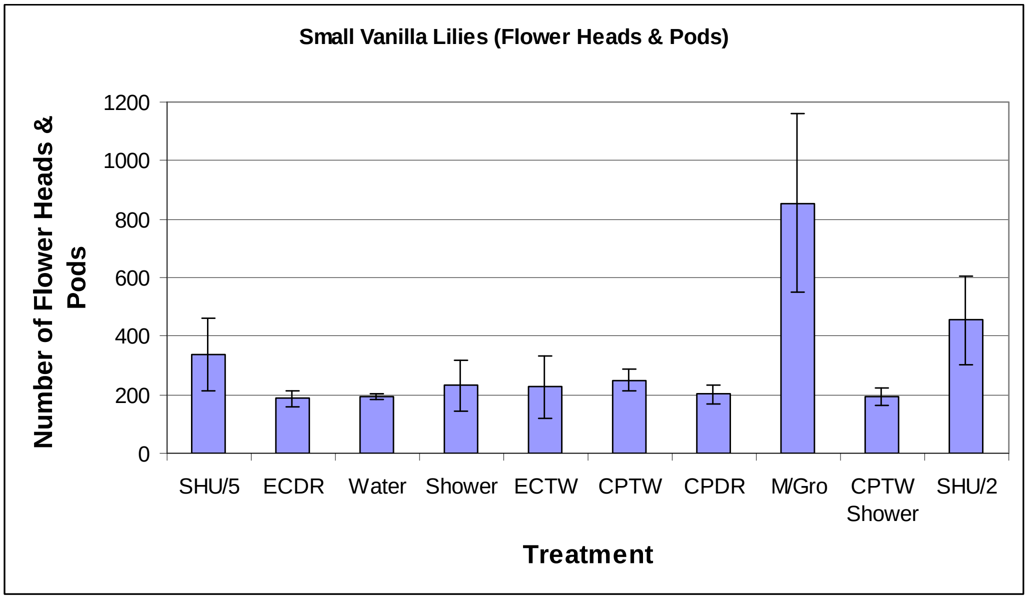

Figure 9.10 – Small Vanilla Lilies (Flower Heads & Pods) **–** Average number of Flower Heads & Pods per Pot per Treatment (Error bars indicate one Standard Deviation)

9.3.2 Dry weights of the foliage

The overall pattern of results for the dry foliage weights is similar to that for the number of flower heads but the difference between the highest and lowest results is reduced. The results are shown in Figure 9.11, and the following observations were made:

- The average dry weights of foliage per pot produced by the seven treatments Water, ECDR, Shower, ECTW, CPTW, CPDR, and CPTW Shower, ranged from 4.9g (ECDR) to 6.1g (CPTW), however the difference between all seven results was not statistically significant ($p = 0.735$).
- M/Gro treatment gave the highest average foliage dry weight per pot of 15.1g, followed by SHU/2 (9.5g), and SHU/5 (8.08g). Difference between the three results was statistically significant ($p = 0.018$).
- M/Gro and Water results indicate that the use of the plant food increased the average foliage dry weight by 201%.
- SHU/2 produced 70% more foliage dry weight than Shower ($p = 0.06$), and SHU/5 produced 45% more dry foliage than Shower ($p = 0.047$).

- Total wash CPTW averaged 11% more foliage dry weight than deep rinse CPDR ($p = 0.463$), and ECTW averaged 15% more than ECDR ($p = 0.465$).
- Phosphate containing CPTW averaged 8.3% more foliage dry weight per pot than phosphate free ECTW ($p = 0.655$).

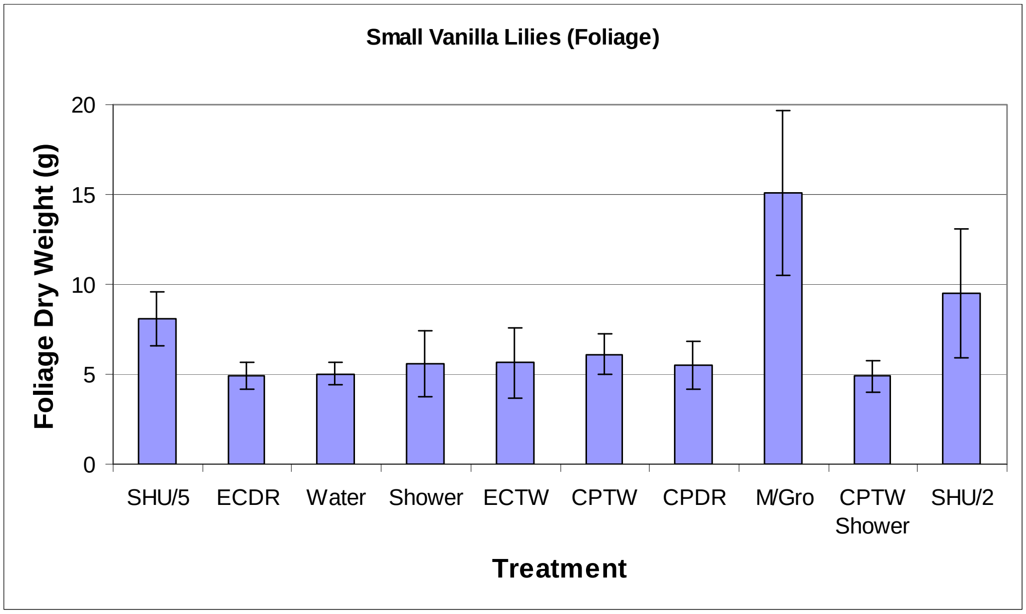

Figure 9.11 – Small Vanilla Lilies (Foliage) – Average Dry Weights (g) of Foliage per Pot per Treatment (Error bars indicate one Standard Deviation)

9.3.3 Dry weights of roots

Small Vanilla Lilies had tuberous roots which did not respond to the treatment solutions in the same way as the number of flower heads and foliage dry weights did. The results obtained by weighing the dry roots are shown in Figure 9.12, and the following observations were made:

- The average dry weight of roots per pot produced by all the ten treatments used in this experiment ranged from 3.32g (CPTW Shower) to 4.78g (M/Gro), however the difference between all ten treatments was not statistically significant ($p = 0.242$).
- The next three heaviest dry root weights after M/Gro were produced SHU/2 (4.38g), Shower (4.36g), and SHU/5 (4.15g).

- Comparison of M/Gro and Water results indicated that the use of the plant food increased the average dry root weight by 23%.
- Shower, SHU/2, and SHU/5 treatment results indicated that the addition of 0.5% v/v urine to shower water increased the average dry root weight per pot by 0.5%, while 0.2% v/v urine produced a 5% decrease in dry root weight.
- Total wash CPTW produced the same average dry root weight per pot as deep rinse CPDR, whereas deep rinse ECDR produced 3.5% more dry root weight than total wash ECTW.
- Phosphate free ECTW produced 4.1% more dry root weight than CPTW.

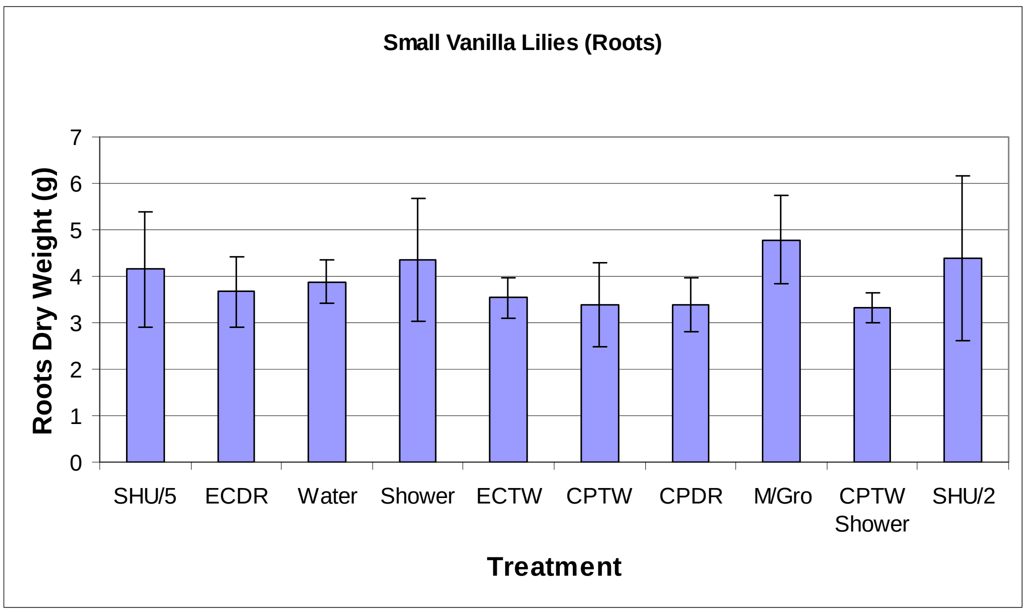

Figure 9.12 – Small Vanilla Lilies (Roots) **-** Average Dry Weights (g) of Roots per Pot per Treatment (Error bars indicate one Standard Deviation)

9.3.4 Stem Length Sum (SLS)

The Small Vanilla Lilies initially grew as rosettes of slender leaves however by the start of September most of the plants had started to produce stems which grew quickly, and on which flowers grew. Observation from a distance did not indicate any great deal of difference in the heights of the fully grown stems resulting from the various treatments. The Stem Length Sum (SLS) for each pot was determined by measuring the longest stem from each of the four plants in a pot, and then adding the four lengths together. The results are displayed in Figure 9.13, and the following observations were made:

- The average SLS results produced by all the ten treatments used in this experiment ranged from 1062 mm (Water) to 1226 mm (M/Gro), however the difference between all ten treatments was not statistically significant ($p = 0.184$).
- SHU/5 (1214 mm), SHU/2 (1158 mm) and CPTW (1154 mm) produced the next three largest SLS results and all the others except Water gave SLS results above 1100 mm.
- M/Gro produced a 15% larger average SLS than Water.
- SHU/2 and SHU/5 results were 4% and 9% respectively larger than due to Shower.
- Total wash CPTW was 4.7% larger than CPDR, and ECTW was 1.5% larger than ECDR.
- CPTW produced 1.9% larger SLS than ECTW.

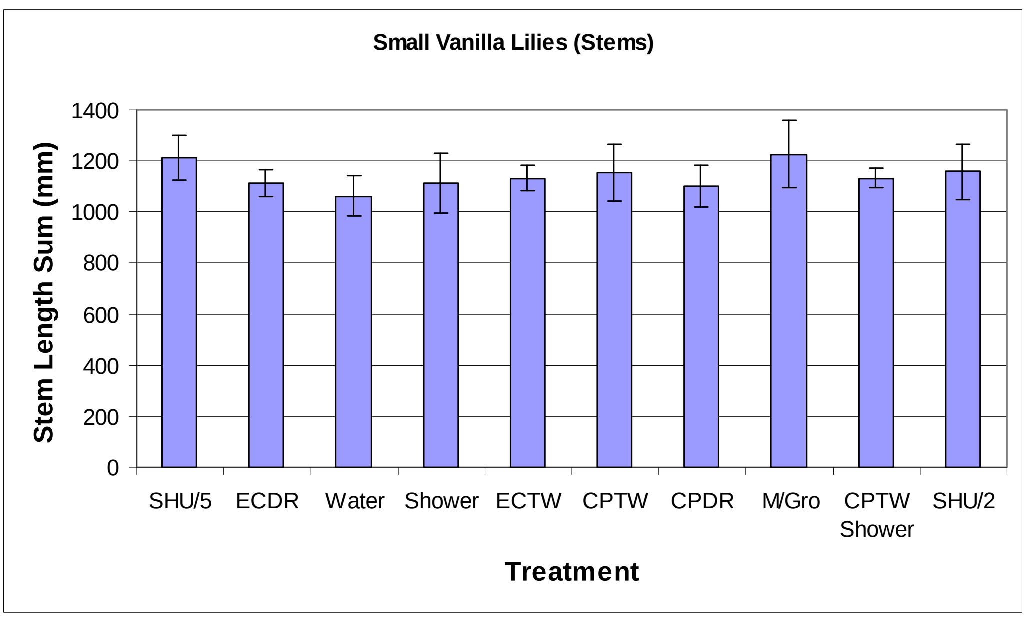

Figure 9.13 – Small Vanilla Lilies (Stems) **–** Average Stem Length Sums (mm) per Pot per Treatment (Error bars indicate one Standard Deviation)

9.3.5 Photographs of four harvested Small Vanilla Lilies

To illustrate the effect of using 0.5% v/v urine in shower water on Small Vanilla Lilies it was decided to present four photographs rather than two, because with the Small Vanilla Lilies there was far greater variation between results than obtained with the Scaly Buttons, and two photographs would not show the extent of this variation. The plotted results for the average number of flower heads and pods (Figure 9.10), and foliage dry weights (Figure 9.11) show that the Standard Deviations tend to somewhat overlap between SHU/2 and Shower, and between Shower and SHU/5. These types of overlaps do not occur with the flower head and foliage weight results for the Scaly Buttons (see Figure 9.1 and Figure 9.2).

Photographs (Figure 9.14 and Figure 9.16) are of SHU/2 treated plants from pots M18 and Q20 respectively, whereas (Figure 9.15 and Figure 9.17) are of Shower treated plants from pots M6 and Q8 respectively. Comparison of plants from pots M18 and M6 shows less difference in total flower heads and foliage mass, than that of plants from pots Q20 and Q8.

Figure 9.14 – Small Vanilla Lilies (Harvested) grown with SHU/2 treatment – (M18)

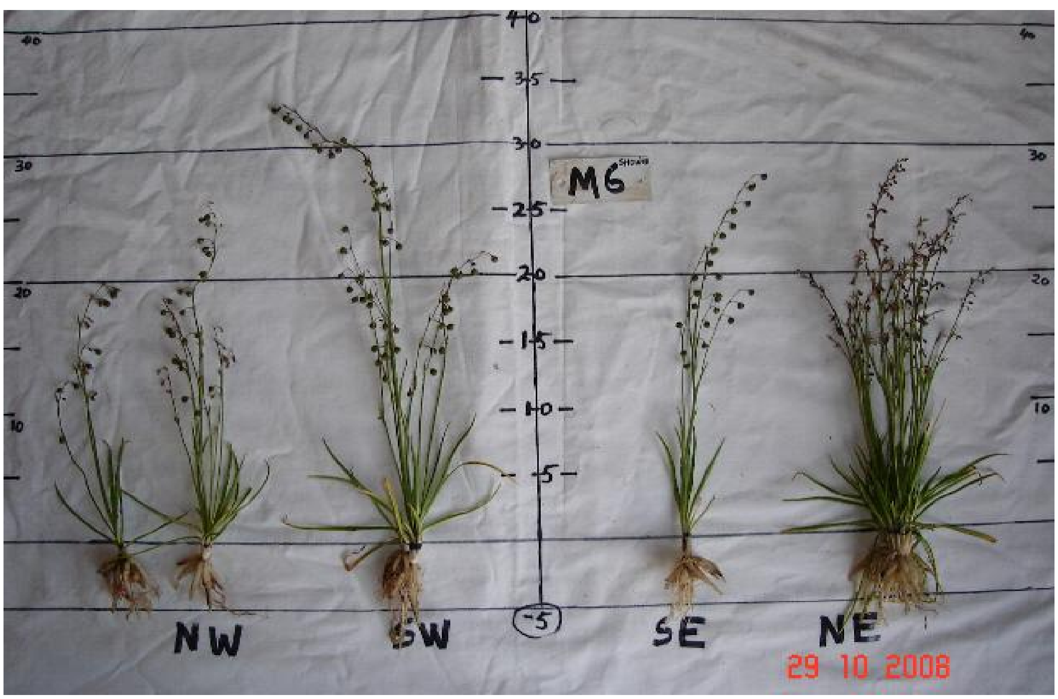

Figure 9.15 – Small Vanilla Lilies (Harvested) grown with Shower treatment – (M6)

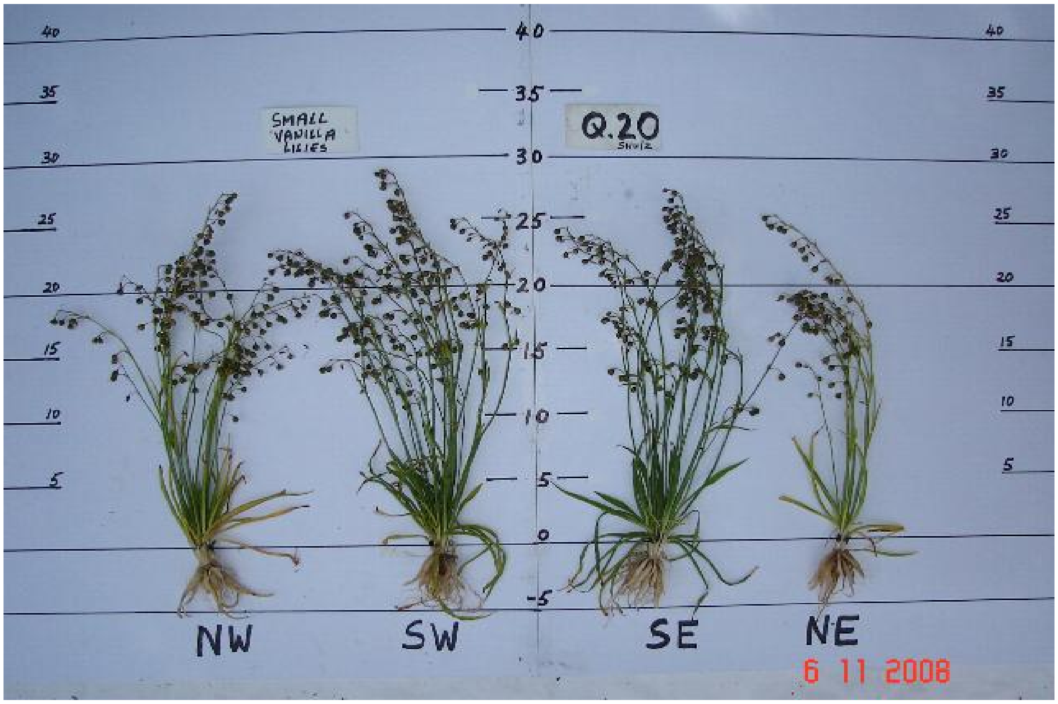

Figure 9.16 – Small Vanilla Lilies (Harvested) grown with SHU/2 treatment – (Q20)

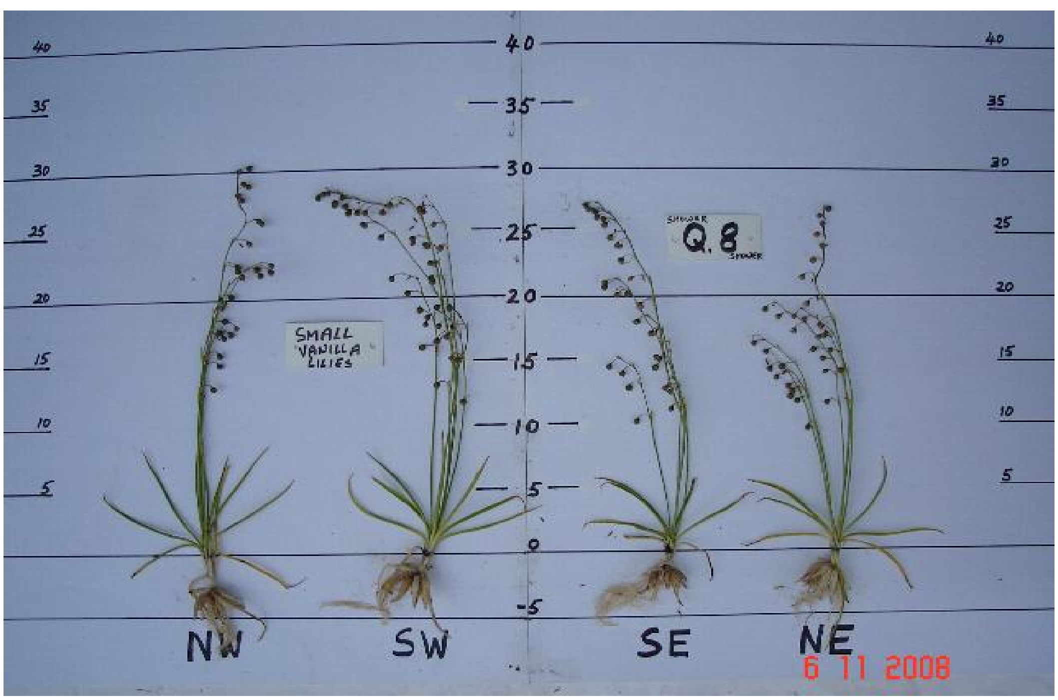

Figure 9.17 – Small Vanilla Lilies (Harvested) grown with Shower treatment – (Q8)

In Figure 9.16 it can be seen that the plant which was situated in the North West (NW) position of the pot was actually two plants. Both plants were included in the total results for the pot. The Small Vanilla Lilies had intertwined multiple plants in several pots.

9.3.6 Measurements of Small Vanilla Lilies during early growth

The Small Vanilla Lilies initially grew as rosettes of long narrow leaves. The Leaf Length Sums (LLS) for each pot were determined by measuring the longest leaf of each plant in a pot and adding the four measurements together. The LLS measurements were determined on three occasions during the growing period, with the last being when stems started to grow from the middle of the rosettes of leaves. The stems on which the flower heads developed were measured at harvest, and the Stem Length Sum (SLS) results have already been reported in Section 9.3.4.

Application of the treatment solutions began on May 22, 2008 and the first set of measurements were carried out a week later. Most of the average LLS results were similar with eight of them ranging between 200 mm and 227 mm. The two lowest results were obtained with Water (182 mm) and SHU/5 (195 mm) treatments.

After 6.5 weeks of treatment the average LLS results due to M/Gro (396 mm) and SHU/2 (344 mm) treatments stood out from the rest. The remaining eight treatments averaged results ranging from 295 mm to 321 mm, with Water (313 mm) no longer giving the lowest result. Average LLS result for M/Gro was 26.5% higher than for Water, SHU/2 was 7% higher than Shower, but SHU/5 was about 4% lower than Shower.

After a further 7.5 weeks of treatment the final measurements showed that:

- M/Gro (440 mm) and SHU/2 (373 mm) gave the largest LLS results. The next closest were due to CPDR (339 mm), SHU/5 (337 mm), and Shower (334 mm).
- The M/Gro result was now 34.5% higher than that for Water.

- SHU/2 was 11.6% higher than Shower, and SHU/5 had caught up with Shower.
- The average LLS result for deep rinse CPDR had now overtaken CPTW by about 3%, while deep rinse ECDR had slightly higher average LLS results than ECTW on all three measuring occasions.

The Average LLS results for the three measuring occasions are shown in Figure 9.18.

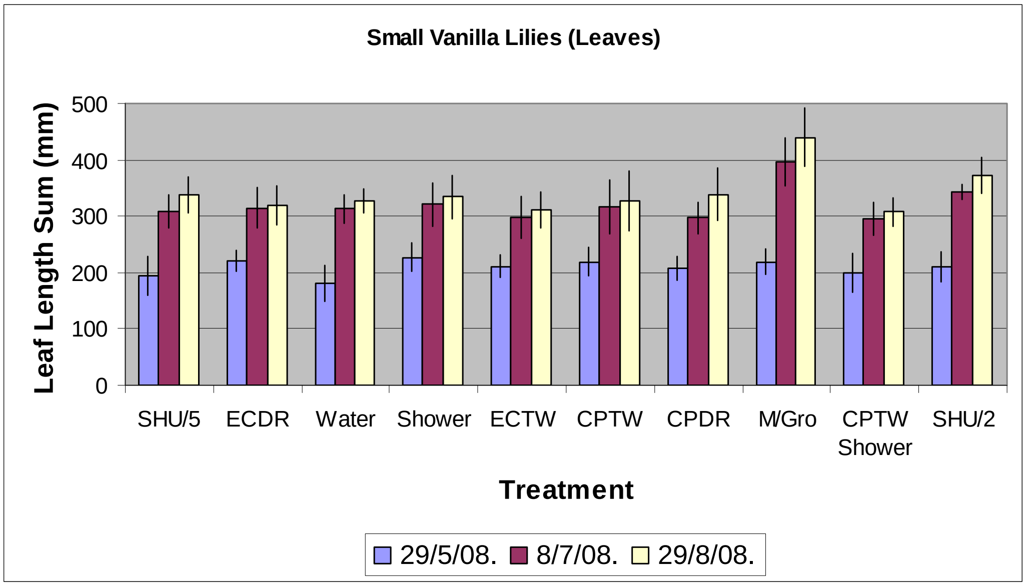

Figure 9.18 – Small Vanilla Lilies (Leaves) – Average Leaf Length Sums (mm) per Pot per Treatment as determined on three occasions (Error bars indicate one Standard Deviation)

9.3.7 Early photographs of two Small Vanilla Lilies

The following photographs (Figure 9.19 and Figure 9.20) of Small Vanilla Lilies in a M/Gro treated pot M14 and a Water treated pot M8 were taken after 6 weeks of applying Miracle-Gro® all purpose plant food (M/Gro). It can be seen that M/Gro treatment was producing some longer leaves than the Water treatment at this early stage.

Figure 9.19 – Small Vanilla Lilies grown with M/Gro treatment (Early growth)

Figure 9.20 – Small Vanilla Lilies grown with Water treatment (Early growth)

The following Chapter 10 discusses the results that have been presented in this Chapter 9.

Chapter 10: Discussion & Conclusions for Native Flowers

10.1 Introduction

Of the four methods used to evaluate the response of both native flower species to the applied treatments, the counting of flower heads and the determining of the foliage dry weights were considered to be the most reliable. These two methods gave the widest spread of growth results between the best performing treatments and the worst performing. The stem length sum (SLS) method gave reasonable results for the Scaly Buttons, but with a narrower separation of results produced by the treatments, than obtained with the flower heads or foliage methods. The narrower range was thought to be partly due to the SLS method not taking into account factors such as thickness of stems or number of stems, both of which were accounted for with the foliage dry weight method. Measurement of stem lengths or leaf lengths were each useful as measurements of growth during the highest growth period, when flowers had not formed, or foliage weights and root weights could not be determined. Determining the dry weights of the roots was considered to be the least reliable indicator of any differences in growth between the treatments. The main problem with using root biomass for the Scaly Buttons was in getting all the roots out without leaving some long thin fibrous pieces behind. With the Small Vanilla Lilies there were sometimes multiple intertwined tuberous roots, or sometimes what appeared to be intertwined old roots, which also presented some uncertainty in determining the correct dry weights.

Table 10.1 below provides a summary of the growth responses of the two native flower species Scaly Buttons and Small Vanilla Lilies, to the greywater treatments that were used in these experiments, as compared to the growth due to water only. Further discussion of the effects of the greywaters on the two native flower species then follows.

Table 10.1 – Summary of native flower growth responses to the greywater treatments – Growth rates are compared to the growth due to water only.

Treatment	Response	Comments & Conclusions
Water Only	Control	Point of comparison for all other treatments
CPDR – (Cold Power® Deep Rinse) ECDR – (Earth Choice® Deep Rinse) CPTW – (Cold Power® Total Wash) ECTW – (Earth Choice® Total Wash) Shower water. CPTW Shower (50/50 blend of CPTW and Shower water).	Overall the Small Vanilla Lilies tended to produce slightly more flowers and foliage with these six treatments than with water alone. By comparison the reverse occurred with the Scaly Buttons except when treated with CPTW. None of the differences in growth results between the six greywaters and water were statistically significant, for each of the species of flowers Phosphate containing CPTW did not produce significantly greater growth than non phosphate ECTW.	All of the treatments can be used as a source of water on the native flowers Scaly Buttons and Small Vanilla Lilies. Some of the phosphate may not have been available to the native flowers by being locked up in the soil, or some of the complex phosphate may not have hydrolysed to a plant usable form.
M/Gro – (Miracle-Gro® All Purpose plant food)	Controlled addition of nutrients. M/Gro produced significantly more foliage and flowers in each of the flower species, and significantly greater root weight in the Scaly Buttons than produced by water, any of the six treatments without added urine, and the two treatments with added urine i.e. SHU/2 and SHU/5.	Added as a solution in water every 14 days. M/Gro produced very good growth in both the Scaly Buttons and the Small Vanilla Lilies. No phosphate toxicity in the plants was evident under the experimental conditions. M/Gro or similar fertiliser would appear to be safe to use to satisfactorily grow Scaly Buttons and Small Vanilla Lilies
SHU/2 – (Shower water with 0.5% v/v urine) SHU/5 – (Shower water with 0.2% v/v urine)	Overall in both flower species SHU/2 and SHU/5 produced significantly more foliage and flowers than water and any of the six treatments without added urine. SHU/2 also produced more foliage and flowers than SHU/5, but considerably less foliage, flowers, and Scaly Buttons root weight than produced by M/Gro.	Urine-containing water treatments were added every watering session. Urine can be added to shower water or other greywater to promote growth in the Scaly Buttons and the Small Vanilla Lilies. The growing season was mainly during the cooler months which required less frequent watering. It is possible that the plants may have tolerated up to 1% v/v urine in Shower water.

Miracle-Gro® All Purpose plant food (M/Gro) produced the greatest growth in both species of native flowers, followed significantly behind by the urine containing treatments SHU/2, then SHU/5. The treatments M/Gro, SHU/2, and SHU/5 each produced significantly more flower heads, and greater foliage dry weights with both species of native plants, than the seven treatments which did not contain added urine or plant food (i.e. ECDR, Water, Shower, ECTW, CPTW, CPDR, and CPTW Shower), and with the Scaly Buttons also larger stem length sums. The growth results produced by the seven treatments without added urine were statistically similar for both of the two native flower species.

10.2 Discussion on results with the native flowers

The following discussion is based on the average results obtained during the experiment, and as an aid to this discussion Table 10.2 has been prepared.
The table below shows the percentage increase or decrease in average results calculated for the number of flower heads, dry weights of foliage and roots, and stem length sums, when comparing two of the named treatments applied to the native plants. The percentages show how the first named treatment performed against the second named treatment, e.g. for *SHU/2 v Shower* the average number of Scaly Buttons flower heads per pot **increased** by 472% when using SHU/2 treatment compared against Shower treatment. With *CPDR v Water* the average number of Scaly Buttons flower heads **decreased** by 26% when using CPDR treatment compared against Water treatment.

Table 10.2 – Native Flowers – Comparisons of Percentage Increase (black) or Decrease (-red) in Average Growth Results between selected Treatments taken Two at a Time, for both Scaly Buttons (SB) and Small Vanilla Lilies (SVL)

	Flower Heads		Foliage		Roots		SLS	
COMPARISON	SB	SVL	SB	SVL	SB	SVL	SB	SVL
SHU/2 v Shower	472	96	397	70	24	0.5	43	4
SHU/5 v Shower	170	46	161	45	0.8	-5	29	9
SHU/2 v SHU/5	112	34	90	18	23	6	11	-5
M/Gro v SHU/2	73	88	88	59	110	9	26	6
M/Gro v SHU/5	267	153	258	87	158	15	39	1
M/Gro v Water	926	338	736	201	145	23	93	15
SHU/2 v Water	491	133	344	90	17	13	53	9
SHU/5 v Water	179	73	133	61	-5	7	38	14
CPTW v CPDR	77	24	46	11	19	0	10	5
ECTW v ECDR	69	21	36	15	30	-4	23	2
CPTW v Water	31	28	13	22	-13	-13	13	9
CPDR v Water	-26	3	-23	10	-26	-13	3	4
ECTW v Water	-16	16	-5	12	-0.6	-9	15	7
ECDR v Water	-50	-4	-30	-2	-24	-6	-6	5
Shower v Water	3	19	-11	11	-6	12	7	5
CT Show v Water	-9	-0.5	-3	-2	-35	-14	4	7

(CT Show = CPTW Shower)

10.2.1 Effect of greywater on growth of native flowers

Each of the species of native flowers were treated with greywater from the time new growth became evident until the plants were harvested, which was 36 weeks for the Scaly Buttons, and 23 weeks for the Small Vanilla Lilies. As a result of the experiment the following observations were made:

- The greywaters or treatments used in the experiment did not cause harm to the Scaly Button or Small Vanilla Lilies, as judged by comparison of growth or flowering rates with the water alone treatment.
- There were no statistically significant differences between the growth produced by Water, and the six types of greywaters which did not contain

added urine as a source of nutrients i.e. CPTW, CPDR, ECTW, ECDR, Shower, and CPTW Shower.

- The two treatments which contained added nutrients in the form of urine i.e. SHU/2 and SHU/5 produced increased growth in both species of native flowers.
- Both species of native flowers thrived and produced the largest growth with regular application of the 13.1% phosphorus containing Miracle Gro® All Purpose plant food.

It is therefore concluded that greywaters as used in this experiment (both with and without urine), can be safely used on the native flowers Scaly Buttons and Small Vanilla Lilies, and that compared against Water, the urine free greywaters will not cause significant increases or decreases in growth of Scaly Buttons or Small Vanilla Lilies. Although greywaters such as CPTW contain some plant nutrients it appears the levels of nutrients in the greywaters used were insufficient to significantly boost the growth of the native plants, unless urine was added or a plant fertiliser was applied. The two species of native plants did not show decreased growth when treated with the phosphate containing laundry water CPTW.

10.2.2 Effect of using Miracle Gro® All Purpose plant food

M/Gro v Water, *M/Gro v SHU/2*, and *M/Gro v SHU/5*

For both native flower species, the addition of 200 ml Miracle-Gro® plant food solution every 14 days produced very large increases in the number of flower heads and foliage dry weights, when compared against Water and the treatments which did not contain urine. The average percentage increases were considerably larger for the Scaly Buttons than for the Small Vanilla Lilies, with the improvement in above ground growth also noticeable from an early stage. Stem length sums and root dry weights increased with M/Gro treatment, especially for the roots of the Scaly Buttons which had dry weights almost 2.5 times heavier than those produced by Water after 36 weeks of growth.

M/Gro also produced significantly greater growth than the next highest growth producing treatments SHU/2 and SHU/5, which respectively contained 0.5 % v/v urine and 0.2 % v/v urine, as a form of fertiliser.

The results show that the native flowers Scaly Buttons and Small Vanilla Lilies thrived when regularly fed with a commercial plant fertiliser containing 13.1% phosphorus. Phosphorus sensitivity or toxicity did not appear to be a problem for either species, as judged by their growth under the experimental conditions. The conclusion is that the two Australian native flower species Scaly Buttons and Small Vanilla Lilies can tolerate phosphorus, and can be successfully grown with the aid of a soluble plant fertiliser having a N:P:K ratio of 15:13.1:12.4.

10.2.3 Effect of adding urine to shower water

SHU/2 v Shower, SHU/5 v Shower, and *SHU/2 v SHU/5*

The addition of urine to shower water increased the average number of flower heads, foliage dry weights, and stem lengths for both species of native flowers. The plants were grown over the cooler months so did not always require to be watered everyday. From results shown in Figures 3.1 and 3.2 it was calculated that 200 ml of M/Gro solution averaged approximately 62 times more phosphorus and 3.5 times more nitrogen than 300ml of SHU/2. Nitrogen was therefore the main nutrient added to the plants with SHU/2 and SHU/5 treatments. Even if watering was carried out everyday the total amount of phosphorus added to the plants with SHU/2 or SHU/5 treatments would be small compared to the amount from one 200 ml application of M/Gro every 14 days.

The Scaly Buttons responded better to the urine, displaying considerably larger percentage increases in flower heads and foliage weights, than the Small Vanilla Lilies. Analysis of Scaly Buttons results showed that all differences between shower water and shower water with added urine were statistically significant. By comparison, analysis of Small Vanilla Lily results showed there were no statistically significant differences in the number of flower heads or foliage dry weights between SHU/2 and SHU/5 treatments, nor was there a significant difference in the number of flower heads between SHU/5 and Shower treatments. The Scaly Buttons also showed statistically significant increases in flowers and foliage weights when the urine content was increased from 0.2% v/v (SHU/5) to 0.5% v/v (SHU/2), whereas the increases shown by the Small Vanilla Lilies were not statistically significant. SHU/2 also produced a moderate increase (24%) in the average dry weight of the Scaly Buttons roots, when compared against Shower treatment.

The conclusion is that shower water containing up to 0.5 % v/v urine was not harmful to the two species of native flowers Scaly Buttons and Small Vanilla Lilies, and that the urine was beneficial to the above ground growth of both species of plants, with the effects on growth being noticeable from early in the growing period. It appears that shower water containing up to 0.5% v/v urine can be successfully used to grow Scaly Buttons and Small Vanilla Lilies during periods of water use restrictions, and also at other times. Trials under a variety of conditions (e.g. range of soil types, wider range of detergents, longer time frames) are needed to further verify these conclusions.

10.2.4 Comparison of Total Wash waters with Deep Rinse waters

CPTW v CPDR and *ECTW v ECDR*

Even though the deep rinse waters CPDR and ECDR were produced separately from the total wash waters CPTW and ECTW, the deep rinse waters could be considered to basically be dilute versions of their respective total wash waters, with regards to the laundry detergent they contained. CPTW and CPDR were derived from phosphorus containing Cold Power® laundry powder, whereas ECTW and ECDR resulted from phosphorus free Earth Choice® laundry liquid. Both total wash waters produced greater average numbers of flower heads, greater average foliage dry weights, and greater average stem length sums in both species of plants, than their corresponding deep rinse waters. The Scaly Buttons for example showed increases in the average number of flower heads by around 70%, and the foliage dry weights by around 40%, with both total wash waters. Despite these differences in average results, statistical analysis showed that there were no significant differences between the growth produced by the total wash waters and their respective deep rinse waters.

Judging by the statistical analysis and the wide variations in replicate results, such as the number of Scaly Buttons flower heads produced by CPTW, CPDR, ECTW, and

ECDR the conclusion is that the growth differences that resulted from total wash and deep rinse waters may be due to individual plant variations. Additional experiments, using a higher number of replicates, might reveal whether or not the differences in averages of growth between the various greywater treatments are significant, or just a result of natural variation.

10.2.5 Comparison of urine free Greywaters with Water

CPTW v Water, CPDR v Water, ECTW v Water, ECDR v Water, Shower v Water, and *CPTW Shower v Water*

In this context *urine free* means that no urine was added to these greywaters, although all of these may have at times contained small traces of urine or urea, which might have been extracted from the items being washed. Statistical analysis comparing Water, CPTW, CPDR, ECTW, ECDR, Shower, and CPTW Shower showed no significant differences between the average numbers of flower heads, foliage and root dry weights, and stem length sums, between any of these water types on both species of native flowers. The many negative percentage results, albeit some small, obtained under the experimental conditions and as shown in Table 10.1 above, may indicate that water could produce some better growth results with the native plants, than produced by some of the urine free greywaters, although this was not able to be confirmed by statistical analysis. The overall average growth results for Scaly Buttons and Small Vanilla Lilies are summarised in the following Figure 10.1 and Figure 10.2.

In Figure 10.1 it can be seen that the urine free greywaters, with the main exception of CPTW, produced Scaly Buttons with decreased flower heads, foliage and root weights, when compared with Water, while the lengths of the longest stems increased. The differences in growth however were not statistically significant to conclude that the differences were not due to plant variations alone.

Figure 10.2 shows that the urine free greywaters (except for ECDR and CPTW Shower) produced Small Vanilla Lilies with increased flower heads, foliage dry weights, and stem length sums, while the root dry weights were decreased except with Shower water. As with the Scaly Buttons the differences in growth were not statistically significant to conclude that they were not due to plant variations alone.

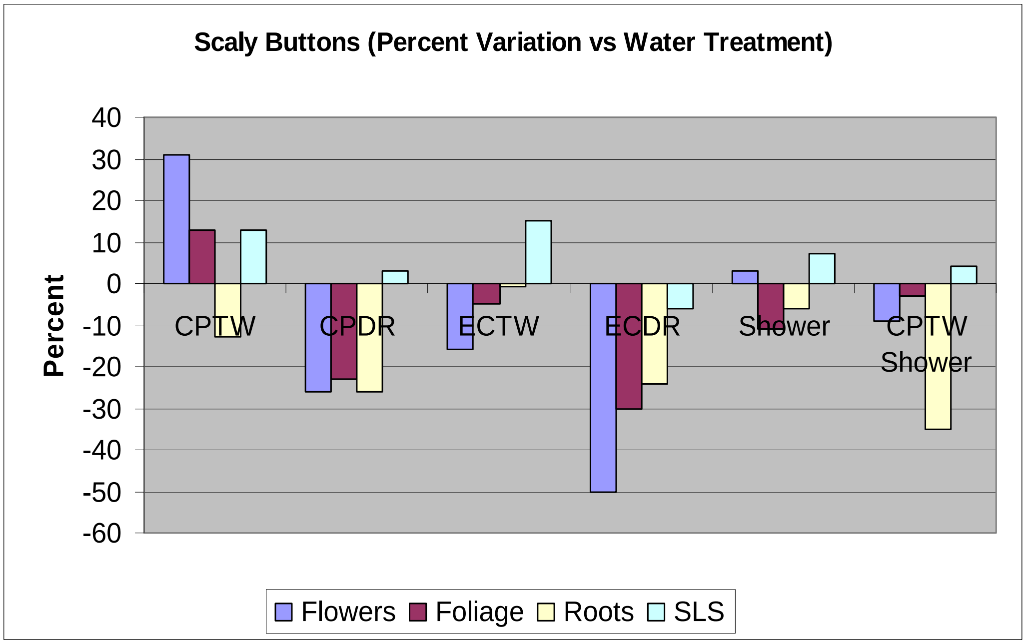

Figure 10.1 – Scaly Buttons **–** Percent Increase or Decrease in Average Results calculated for urine free greywaters compared against Water treatment

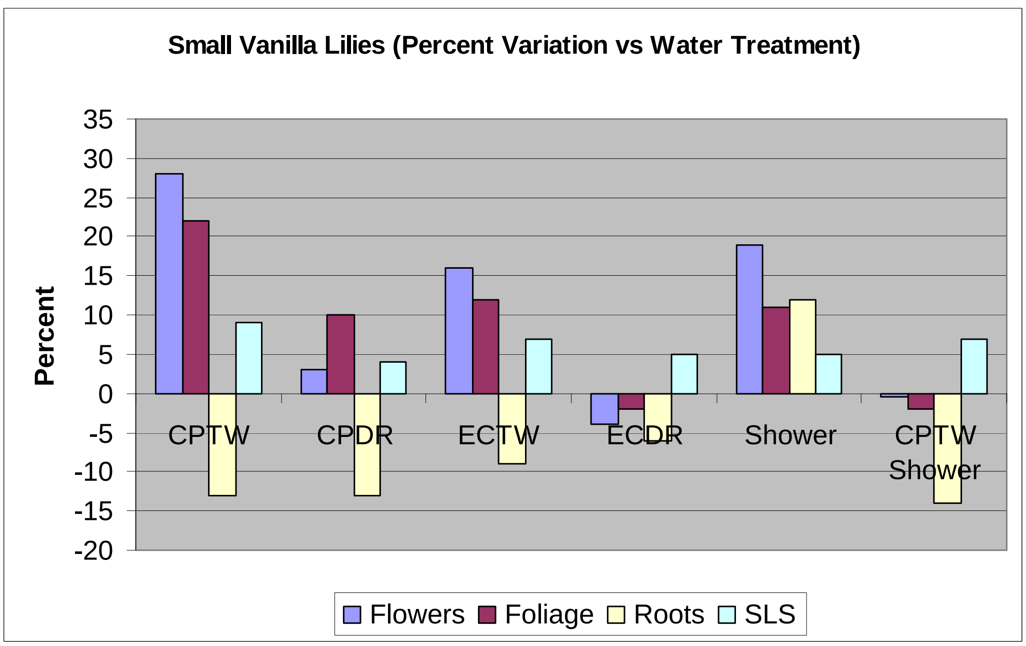

Figure 10.2 – Small Vanilla Lilies **–** Percent Increase or Decrease in Average Results calculated for urine free greywaters compared against Water treatment

10.2.6 Comparison of root, foliage, and flower head results

The following Figure 10.3 shows a comparison of the dry root and foliage weights, and 10% of the flower head count results for the Scaly Buttons. Ten percent of the actual flower head count was used so that the plotted results would not be too large to be compared against the results for the roots and foliage on the same bar graph. A similar plot was made for the Small Vanilla Lilies (Figure 10.4), except that only 2% of the flower head count was used.

The Scaly Buttons had long fibrous roots and the results show that with the doubling of the dry root weight produced by M/Gro treatment, as compared against SHU/2, the dry weight of the foliage and the flower head count also increased significantly. Comparison of the results produced by SHU/2 and SHU/5 shows a small root weight increase, but a significant doubling of the foliage weight and the flower head count. Comparison of the results for SHU/5 against the seven treatments without added urine shows that although the SHU/5 root weight is not statistically different to the root weights of the seven treatments, the foliage weight and the flower head count produced by SHU/5 increased significantly. The results in Figure 10.3 indicate that for the Scaly Buttons, increased root weight leads to increases in foliage weight and in the number of flowers, but increased foliage weight and flower head count does not necessarily indicate increased root weight.

The Small Vanilla Lilies had compact tuberous roots and the results show that although M/Gro produced 9.1 % heavier roots than SHU/2, there was no statistically significant difference between the dry root weights produced by all of the ten treatments including M/Gro. However M/Gro produced a significantly increased dry foliage weight and flower head count, followed to a lesser extent by SHU/2, and then by SHU/5. The results in Figure 10.4 indicate that for the Small Vanilla Lilies the root weights do not increase significantly, with significant increases in foliage weights and flower head counts.

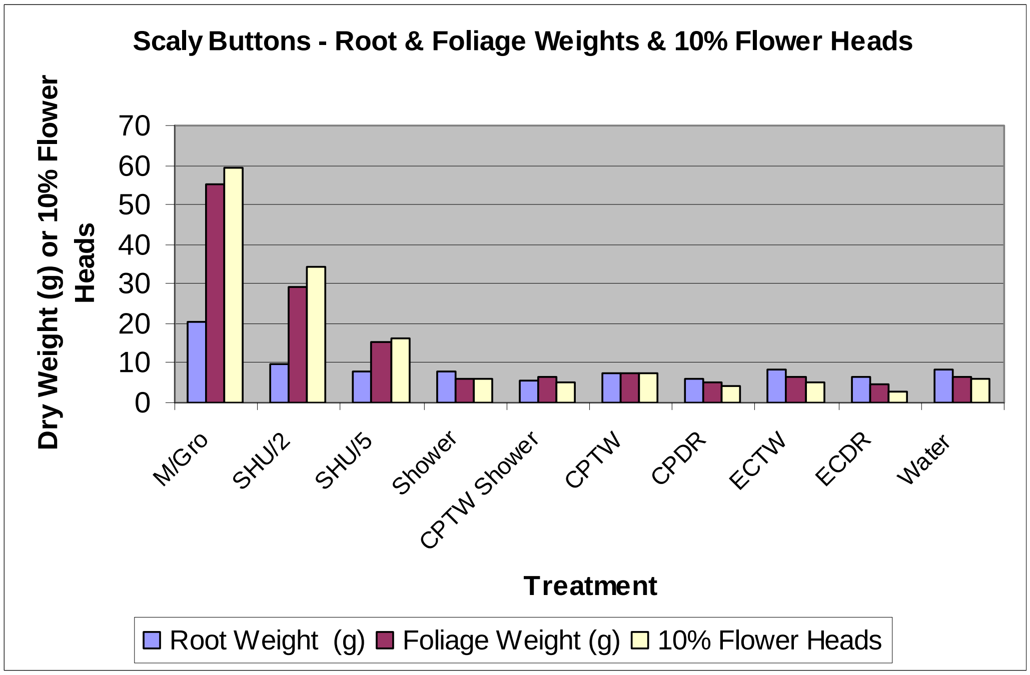

Figure 10.3 – Scaly Buttons – Dry Weights of Roots & Foliage, and 10% of Flower Head count

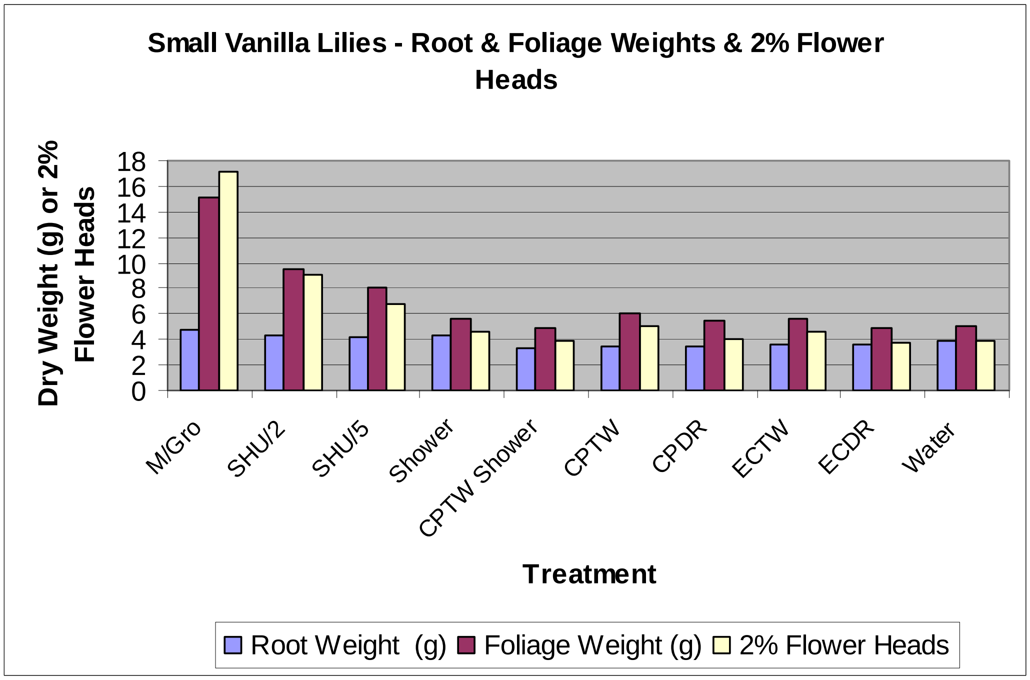

Figure 10.4 – Small vanilla Lilies – Dry Weights of Roots & Foliage, and 2% of Flower Head count

10.3 Comparison of turf and native flower results

The experiments of growing the turf and the native flower specimens with specific greywaters sourced from the shower and the laundry were conducted as separate stand alone trials. Different soils were used because instant turf would in many cases be grown on a bed of sandy loam soil, whereas the native flowers would be grown in a garden bed. The turf specimens were subjected to greywater treatments through the four seasons, whereas the native flowers received greywater treatments during the cooler months. Scaly Buttons for 36 weeks from April 9, 2008 and Small Vanilla Lilies for 23 weeks from May 22, 2008. Some of the greywaters that were added to the native flowers differed to those applied to the turf species, notably the maximum urine level in shower water was halved to 0.5% v/v. Despite these differences some comparisons on how the turf and native flowers reacted to the treatments can be made. The following Figure 10.5 is compiled from Figure 5.2 (Tall Fescue), Figure 6.3 (Kikuyu), Figure 9.2 (Scaly Buttons), and Figure 9.11 (Small Vanilla Lilies).

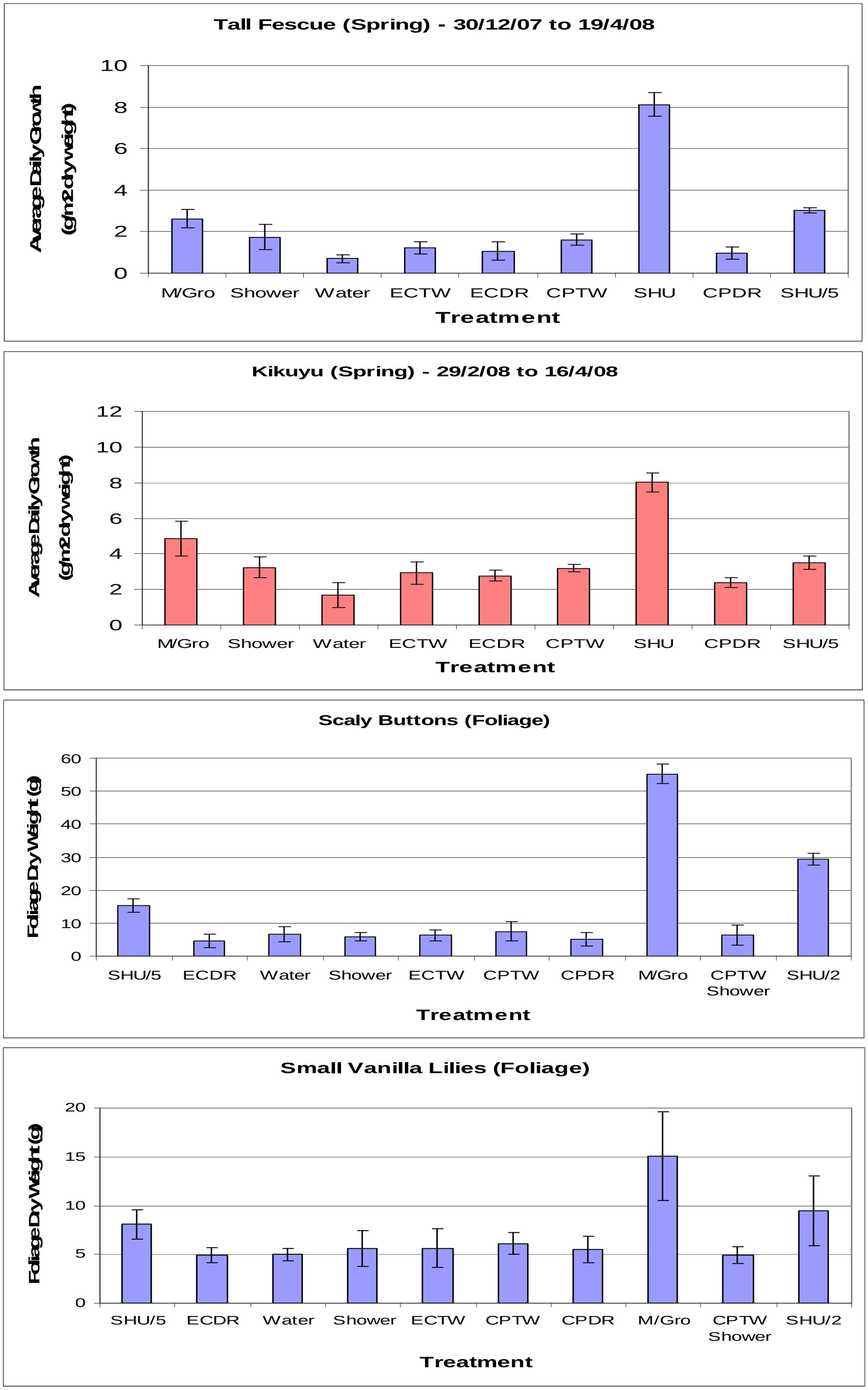

Figure 10.5 – Comparison of Turf and Native Flower results

Sampling of the turf clippings occurred several times during the experimental period which lasted more than a year, and so for each turf species one set of growth results from before winter are shown in Figure 10.5. The destructive sampling of the total foliage from the two native flower species could only be done when the growth experiment was ended. Despite the different sampling periods for these two independent experiments, the similarities between growth results produced by the greywaters and the reference treatments for all four species can be clearly seen. In Figure 10.5 above it can be seen that Miracle-Gro® solution and shower waters containing urine (SHU or SHU/2, and SHU/5) produced more growth in the turf species Tall Fescue and Kikuyu, and in the native flowers Scaly Buttons and Small Vanilla Lilies, than produced by Water and the greywaters which did not contain added urine.

The main difference in the treatments is that shower water containing 1% v/v urine (SHU) was applied to the turf species, and shower water containing 0.5% v/v urine (SHU/2) was applied to the native flowers. Compared to the constant amount of M/Gro that was applied each fortnight to the turf and the native flowers, the turf samples treated with SHU received a greater amount of total urine (and hence nitrogen), than the native flowers treated with SHU/2. The total amount of urine added to the turf was further increased because during the warmer months the watering often had to be done daily, while the native flowers, which grew during the cooler months, often did not need to be watered as frequently. Apart from the differences in growth results due to SHU and SHU/2, as explained above, all four plants were not harmed by the different greywaters and showed growth responses when treated with urine or plant food. The significant increased growth produced by Miracle-Gro® All Purpose plant food in the native flowers, especially the Scaly Buttons, confirms that these two native flowers can be grown successfully with the aid of high phosphorus containing commercial fertiliser.

The maximum level of 0.5% v/v urine was chosen for use on the native flowers after observing that during very hot drying conditions the turf samples appeared to be receiving too much nutrient when SHU was being applied daily. It is now considered that, for the native flowers, reducing the urine level was an error of judgement. It was overlooked that the major growth of the native flowers occurred during the cooler months, when the requirement for watering was less frequent than if the growth occurred during the hot months. With less frequent watering less total urine ends up being added to a plant over an extended period. It therefore may have been feasible to have used the blend SHU (1% v/v urine in shower water) for watering of the native flowers, and this may have produced different results than those observed for these two species.

10.4 Summary

Subject to the experimental conditions under which the Scaly Buttons and Small Vanilla Lilies were grown, the following conclusions are made:

1. All of the greywater types used in the experiments reported here appear to be safe for application to Scaly Buttons and Small Vanilla Lilies.

2. Both species of native plants responded well to shower waters containing urine levels of 0.5% v/v and 0.2% v/v, i.e. levels that could occur if a person urinated while having a shower. The results indicate that urine added to shower water should significantly increase the number of flower heads and foliage weights of the Scaly Buttons and the Small Vanilla Lilies, at least in the first season of growth.

3. The native plants responded extremely well to phosphate containing Miracle-Gro® All Purpose plant food, with no phosphate toxicity being noticed. The results indicate that Miracle-Gro® or similar plant food should produce considerable percentage increases in the number of flower heads and foliage dry weights of both species, and a significant increase in the root dry weights of the Scaly Buttons.

4. The total wash laundry waters produced more flower heads and higher foliage dry weights than their corresponding deep rinse waters, but in general the differences were not statistically significant. The total wash waters contained considerably more extracted soil and laundry compound which may have

contributed to increased growths. Increased numbers of replicates may have revealed some difference in these treatments.

5. The Scaly Buttons tended to respond better to the urine containing treatments, Miracle-Gro®, and the total wash waters than the Small Vanilla Lilies, showing larger percentage increases in number of flower heads, foliage weights, and lengths of longest stems.

6. The Small Vanilla Lilies showed better response to most of the urine free greywaters than to Water, whereas the Scaly Buttons performed better with Water than with most of the urine free grey waters. It is important to note however, that the differences in growth results between Water and the urine free greywaters, for both species of natives, were not statistically significant.

7. When compared against Water all applications of urine free greywaters, except for Shower on Small Vanilla Lilies, produced roots with decreased average dry weights. The differences in root growths were in general not statistically significant, except for Scaly Buttons roots produced by Water treatment compared with CPTW Shower treatment. Whether the measured decreases in root weights will have an effect on plant growth in second or third seasons is unknown.

8. The Small Vanilla Lilies did not show significant increases in the weights of their tubular roots, to match significant increases in foliage weights and the number of flower heads produced. The Scaly Buttons that were fed with Miracle Gro® fertiliser however did show a significant doubling of their fibrous root weights, to match the significant increases in foliage weights and flower head counts.

The following Chapter 11 contains recommendations and suggestions for the use of greywater in domestic and small community facility gardens, and also contains recommendations for future research.

Chapter 11: Recommendations

In this study, all the greywaters used were found not to be harmful to plant growth, but produced equal or better growth compared to Water for the turf species Tall Fescue and Kikuyu, and for the native flowers Scaly Buttons and Small Vanilla Lilies. Over the duration of these experiments, it was found that the greywaters without added urine did not contain sufficient nutrients to sustain good plant growth. That is, nutrients had to be added in the form of Miracle-Gro® plant food or urine to increase growth rates significantly over the Water only controls. It was also found that the two native flower species did not suffer from phosphorus toxicity, but produced very good growth when fed with a plant fertiliser containing 13.1 % phosphorus. The study also determined that for the turf and native flower species, the differences in growth produced by the laundry total wash waters and the laundry deep rinse waters were not statistically significant.

The greywaters used in this study that confirmed no detrimental effects on growth of any of the plant species, included total wash and deep rinse waters which allowed comparison of the effects of phosphate-containing and phosphate-free laundry detergents, shower water containing body wash products, and a couple of blends of these shower and laundry greywaters. Some of the greywaters also contained urine added at levels that could result if a person urinated while having a shower. All of the greywaters contained water and the relevant cleaning agent, plus any soils, fats, and nutrients that the greywaters washed off from human-used clothing, linen, towels, or that were washed off directly from the human body.

The following are recommendations and suggestions for the use of greywater in domestic and small community facility garden watering during dry conditions. The recommendations are based on the assumption, that the various greywater types which can be sourced from a shower or laundry are free from significant contaminants such as bleaches, fabric softeners, hard surface bathroom cleaners, solvents, dyes, and faeces:

1. None of the greywater types used in the study reported in this thesis appeared to be harmful to the turf or native plant specimens for the duration of the trial, which exceeded one year. Most types of untreated greywaters from showers and domestic washing machines therefore appear to be useful sources of water for putting onto lawns and gardens, including onto native plants such as the Scaly Buttons and the Small Vanilla Lilies (subject to any health considerations, which appear to be minimal in Victoria at least for these types of conditions).

2. Over time, gardens receiving greywater will need to have some type of supplementary nutrients added. The experimental trials demonstrated that there were not enough nutrients in the urine free greywaters used, to sustain growth at full growth rates, compared with plants with artificially added nutrients (Miracle-Gro® plant food). This depletion of nutrients was relieved by adding urine to several types of greywater, and so it is expected that the use of another source of nutrients such as a commercial fertiliser will also do the same.

3. Urine in diluted form should be considered as a useful nutrient source to add directly to greywater or to plain water being used on lawns or garden beds. The watering may have to be carried out by subsurface irrigation to meet health requirements, a method of irrigation which should be relatively simple to achieve at community facilities. Urine is an ideal plant fertiliser and has been used for a long time in small-scale edible crop gardening around the world (Jonsson et al. 2004). It does not make practical environmental sense to flush this free plant fertiliser to a treatment plant, and then try to extract the nutrients there, and also to use manufactured fertilisers, or animal manures on domestic and community facility gardens.

4. As a means of conserving potable water supplies the occasional use of greywater for lawns and ornamental gardens should be official policy, even at times when water restrictions do not apply. Not every community facility or household will be set up to use greywater for uses such as toilet flushing, but most will be able to use greywater on lawn or ornamental gardens.

5. The study also found that the frequent use of a commercially available water soluble plant food with a high phosphorus content and an N:P:K ratio of 15.0:13.1:12.4, promoted very good growth in the native flowers Scaly Buttons and Small Vanilla Lilies. Under the experimental conditions no phosphorus toxicity was evident in either species of plants. It is therefore suggested that similar types of fertilisers can be used on the same plants, and possibly on related species, subject to further investigation. It may be prudent to check the effectiveness and safety of application of these types of treatments over a longer timeframe.

As a result of this study the following recommendations for further research are suggested:

1. Research to establish the potential scale of direct use of greywater types and addition of dilute urine, in domestic and community facility locations. The trials here were only conducted on two types of exotic grass and two types of local native plants. The results might reasonably be expected to translate to other types of plants, in other situations, but trials are needed to establish this in more detail.

2. Research to determine whether the use of urine for above ground watering of gardens, at similar dilutions that were used in this project, is potentially more or less dangerous to human health than using animal manures, or animal manure products for gardening purposes.

3. Research to determine whether the growth of the native plants Scaly Buttons and Small Vanilla Lilies (or similar species) will be negatively affected during the second or third year of treatment with the various types of greywaters.

4. Research to determine whether the current concentrate laundry products which have replaced the old style heavily bulked laundry washing powders, and whether the specially promoted ‘greywater safe’ products are significantly less harmful to soil and plants, with long term use.

5. Research to determine whether complex phosphates such as Sodium Tripolyphosphate (STPP) are hydrolysed sufficiently into plant usable phosphates while residing in the pots, or do significant amounts of these phosphates exit the pots before being hydrolysed.

6. Research to determine whether alternate watering with a greywater that contains significant amount of salts, and a greywater that contains very little salts, is less harmful to the soil than always watering with a mixture of the two greywaters.

7. Research to determine whether watering lawn areas with greywater will be a satisfactory biological control method against attack by cockchafer beetles. These often decimate lawns by feeding on roots and underground stems, as occurred at the Flemington race track (Habel 2010). The suggestion by Dahler (2008) is to pour biodegradable detergent over the affected areas to encourage the larvae and beetles to come to the surface, where birds and poultry may pick them off. If biodegradable detergent is found to work satisfactorily then the undiluted greywaters such as CPTW or Shower water may also work.

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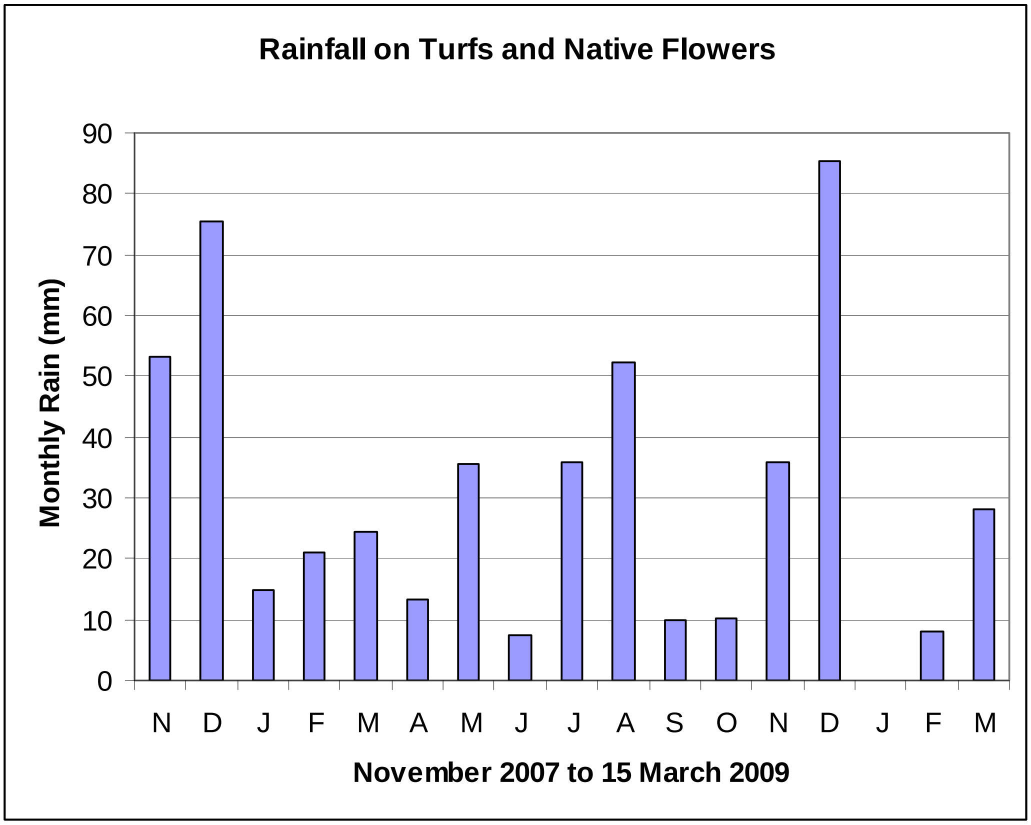
Appendix A

Monthly rainfall on turfs and native flowers

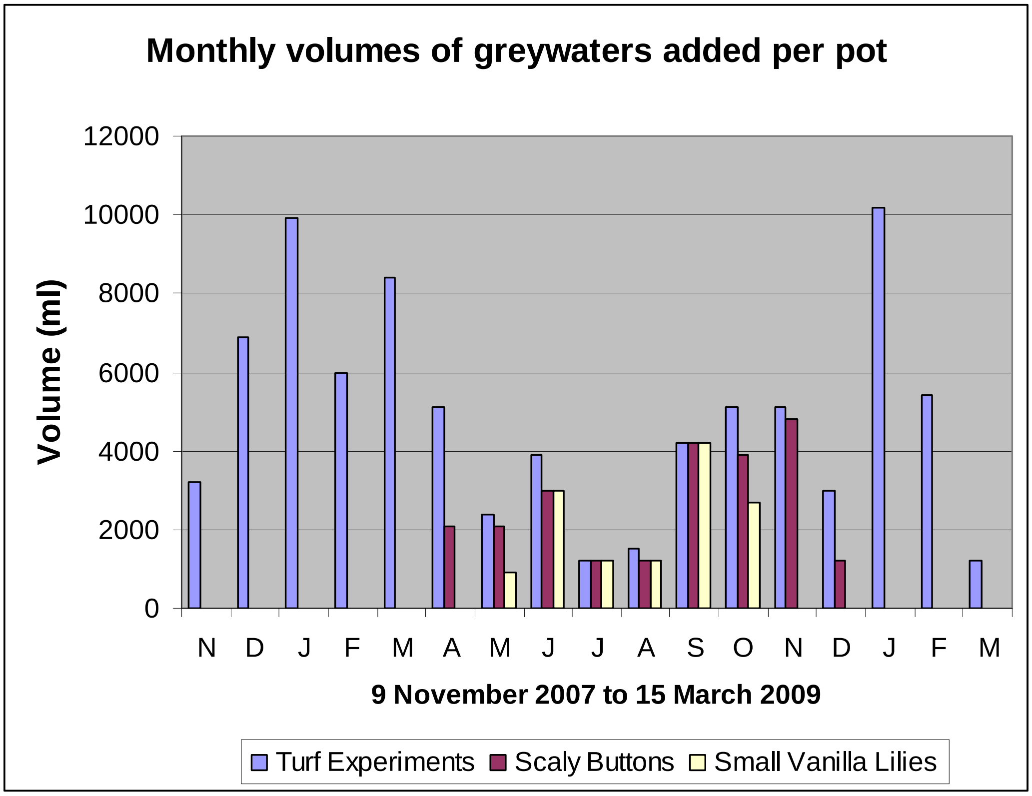


The monthly rainfall that fell on the turf and native flower growing area between 1/11/07 and 15/3/09, was measured by a rain gauge located with a few feet of the samples. The total rainfall over this period was 510.8 mm, and the annual rain for the year January – December 2008 was 346.2 mm. There was no rainfall during the very hot month of January 2009, and very little rain in the first month of winter and during the first two months of spring 2008.

As a comparison the Mean annual rainfall for the thirty year period 1971-2000 that was measured 8 km away at Melbourne Airport, was 556.9 mm, which was 60.9 % greater than the 346.2 mm that fell on the experimental plants during the drought in 2008. The Mean annual rainfall data was obtained from the Bureau of Meteorology using URL http://www.bom.gov.au/climate/averages/tables/cw_086282.shtml

Appendix B

Total monthly amounts of greywater added to plants per pot



Appendix C

Description of the watering treatments used on turfs and native flowers.

- **Water** – Melbourne tap water.
- **M/Gro** – Miracle-Gro® solution. Made by dissolving a 15ml level scoop of Miracle Gro® All Purpose Plant Food per 4 litres of tap water.
- **CPTW** – Cold Power Total Wash. The total collected laundry greywater (wash and rinse cycles) when using Cold Power® Advanced concentrate laundry powder.
- **CPDR** – Cold Power Deep Rinse. The deep rinse only greywater collected when using Cold Power® Advanced concentrate laundry powder. Excludes the waters from the wash and spray rinse cycles of a washing machine.
- **ECTW** – Earth Choice Total Wash. The total collected laundry greywater (wash and rinse cycles) when using Earth Choice® laundry liquid.
- **ECDR** – Earth Choice Deep Rinse. The deep rinse only greywater collected when using Earth Choice® laundry liquid. Excludes the waters from the wash and spray rinse cycles of a washing machine.
- **Shower** – Total collected greywater from bathroom shower when washing body. Only liquid body wash and shampoo products were used and no solid soaps.
- **SHU** – Greywater from bathroom shower as above, with 1% v/v human urine added. Used only on the Turfs.
- **SHU/2** – Greywater from bathroom shower with 0.5% v/v human urine added. Used only on the Native Flowers.
- **SHU/5** – Greywater from bathroom shower with 0.2% v/v human urine added.
- **CPTW SHU (or CPTW & SHU)** – An equal volume blend of CPTW and SHU greywaters. Used only on the Turf samples.
- **CPTW Shower (or CPTW & Shower)** – An equal volume blend of CPTW and Shower greywaters.
- **CPTWU** – Cold Power Total Wash plus Urine (initially 1% v/v and later reduced to 0.5% v/v). Added in the latter stage of the experiment to Spring set of CPTW treated turf samples in an attempt to encourage growth.

- **Shower 0.5U** – Shower plus 0.5% v/v urine. Is actually SHU/2 but labelled differently for a different experiment at the end.
- **CPTWU 0.5U** – Is actually CPTWU which contained 0.5% v/v urine but coded with the extra designation 0.5U because it was being used for a different experiment at the end.
- **ECTW 0.5U** – ECTW plus 0.5% v/v urine.
- **ECDR 0.5U** – ECDR plus 0.5% v/v urine.
- **CPDR 0.5U** – CPDR plus 0.5% v/v urine.
- **Water 0.5U** – Water plus 0.5% v/v urine.