Growth and feed quality of five perennial ryegrass (*Lolium perenne* L.) cultivars under three water treatments

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A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Applied Science

at
Lincoln University
by
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Lincoln University
2017

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Applied Science.

Growth and feed quality of five perennial ryegrass (*Lolium perenne* L.) cultivars under three water treatments

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Growth and feed quality of five perennial ryegrass cultivars were compared under three water treatments: non-stress (field capacity, FC), mid-stress (50% FC) and high-stress (30% FC). There were three New Zealand cultivars (Alto, Bronsyn and Trojan), one Morrocan cultivar (Barberia) and one cultivar from Norway (Norway).

Water stress reduced dry matter production, leaf water content, leaf width, and leaf photosynthetic rate and stomatal conductance of all cultivars by similar amounts. In contrast, water stress increased feed quality (dry matter digestibility and metabolizable energy) but again with no consistent cultivar effect. Averaged over all cultivars, the magnitude of the reduction in dry matter production (44% under high-stress and 21% under mid-stress) was substantially greater than that of the increase in feed quality (2% under mid-stress and 4% under high-stress for dry matter digestibility, 3% under mid-stress and 5% under high-stress for metabolizable energy). For all cultivars, dry matter production and feed quality returned to normal after rehydration of high water stressed plants. It appears that it will be very difficult to develop a cultivar with maintained growth under water stress conditions. However, against this, perennial ryegrass cultivars in general have a strong ability to recover from drought which is an extremely important characteristic in perennial pastures subject to periodic water stress.

Keywords: Perennial ryegrass, *Lolium perenne* L., drought, dry matter production, leaf water content, leaf width, photosynthesis, stomatal conductance, feed quality, dry matter digestibility, crude protein, metabolizable energy .

Acknowledgements

Many thanks to my University supervisor Mitchell Andrews for his patient guidance, support and joyful company; and to Colin Eady my company mentor for his support and trust, offering me this cooperative Masters project between Lincoln University and Agriseeds Ltd; James Morton, my co-supervisor, for his guidance and suggestions when Mitchell was away.

Thanks to the Lincoln Univeristy staff Neil Smith, Simon Hodge, Shuang Jiang, Stephen Stilwell and staff at the Field Research Centre for your help and kindness.

Thanks to the staff at Agriseeds Ltd for your support, and providing the plant material and greenhouse and the touch rugby team, thanks for your team spirit and enthusiasm.

I would like to thank the Callaghan Innovation and AGLS Research Scholarship for funding my Masters studies in New Zealand.

Finally, I want to thank my friends in New Zealand and family in China, your support and encouragement has greatly helped me in the completion of this Masters project.

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Chapter 1

General introduction

1.1 Plant requirements for growth and development

Plants require light, oxygen (O_2), water (H_2O), carbon dioxide (CO_2), mineral nutrients, temperature within a specific range and space to grow and develop (Taiz & Zeiger, 2010; Munns *et al*., 2016). Light (photosynthetically active radiation, PAR 400-700 nm), CO_2 and H_2O are required for photosynthesis, the process that converts solar energy, atmospheric CO_2 and H_2O into carbohydrates and releases O_2, shown in Equation 1.1 (Munns *et al*., 2016).

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{Light}]{\text{Chlorophyll a}} C_6H_{12}O_6 + 6\ O_2 \qquad \text{Equation 1.1}$$

Chlorophyll a is the primary photosynthetic pigment in vascular plants and only it can convert light into chemical energy. The other photosynthetic pigments are chlorophyll b and carotenoids (accessory pigments) (Taiz & Zeiger, 2010; Munns *et al*., 2016). Chlorophyll a and b are universal constituents of vascular plants and express highly characteristic absorption spectra. Chlorophyll b absorbs light and transfers energy to chlorophyll a for photosynthesis. Carotenoids also absorb light and transfer energy to chlorophyll a for photosynthesis and in addition, protect chlorophyll a from photodamage (Young & Britton, 1993; Stahl & Sies, 2004). Plants take up CO_2 from the air through stomata (specialized epidermal guard cells on the surface of leaves and stems) mainly in leaves (Buckley, 2005; Roelfsema & Hedrich, 2005). This CO_2 diffuses into the chloroplast (organelle of photosynthesis) and is reduced to carbohydrate via the reductive pentose phosphate cycle or Calvin cycle (see below). Meanwhile, water is oxidized into O_2 which is released into the air (Munns *et al*., 2016). In addition to photosynthesis, light is involved in photoperiod (daylength), phototropic (bending towards or away from light) and photomorphogenesis (growth patterns) reactions of plants (Hay & Porter, 2006; Mattera *et al*., 2013).

Greater than 95% of all plants are 'C_3' plants. These plants produce a 3 carbon (C) compound on CO_2 fixation (Still *et al*., 2003; Sage *et al*., 2012). Here, CO_2 reacts with 5C ribulose bisphosphate in the presence of the enzyme ribulose bisphosphate carboxylase (Rubisco) to produce two x 3C phosphoglycerate. All the main temperate cereals (e.g. wheat (*Triticum aestivum*) and barley

(*Hordeum vulgare*)), grasses (e.g. perennial ryegrass (*Lolium perenne*) the plant species studied in this project and cocksfoot grass (*Dactylis glomerata*)), grain legumes (e.g. pea (*Pisum sativum*) and lentil (*Lens culinaris*)) and pasture legumes (e.g. white clover (*Trifolium repens*) and lucerne (*Medicago sativa*)) are C_3 plants.

Less than 5% of plants are 'C_4' plants. These plants produce a 4C compound on CO_2 fixation. Here CO_2 reacts with the 3C phosphoenolpyruvate (PEP) in the presence of the enzyme PEP carboxylase to produce a 4C organic acid, commonly malate (Sinclair & Horie, 1989; Sage *et al*., 1999; 2012). This reaction takes place in the leaf mesophyll cells. The organic acid is then transported to the bundle sheath cells where CO_2 is released and fixed via Rubisco as for C_3 plants. C_4 plants are generally tropical grasses: examples are maize (*Zea mays*), sugarcane (*Saccharum officinarum*) and kikuyu grass (*Pennisetum clandestinum*).

As shown in Equation 1.1, water is required for photosynthesis. However, the water used for photosynthesis is only a small proportion of the total water taken up because most water taken up is lost to the atmosphere by transpiration (Kramer & Boyer, 1995; Blum, 2011). Plants extract water from the soil via roots, then transport and translocate this water to the whole plant via long distance xylem transport. After arriving at leaves, a small proportion of the water enters cells but most water is evaporated into the air via stomata: simultaneously, gas exchange occurs in leaves through open stomata. The ratio of water lost to CO_2 taken up is about 300:1 in most plants (Munns *et al*., 2016). Therefore, the dilemma is that plants require more CO_2 to produce more dry matter while rapid gas exchange will result in greater water loss (Stanhill, 1986).

Water transpiration from leaves can reduce the temperature of leaves during hot periods. Also, the phloem, which translocates the products of photosynthesis (usually sugars) and amino acids from mature leaves to growing tissues and organs, including roots, requires water to function. In addition, water provides the driving force for tissue/cell expansion. This internal cellular water pressure is called turgor pressure (Turner, 1997). Water is also the medium in which all plant reactions take place. Generally, water use efficiency (H_2O used per CO_2 fixed) is greater with C_4 plants than C_3 plants (Sage *et al*., 2012; Busch *et al*., 2013). This is because PEP carboxylase has a higher affinity for CO_2 in comparison with that for Rubisco and thus C_4 plants can have a high photosynthetic rate with a small stomatal aperture/high stomatal resistance. C_3 plants show very low photosynthetic rates with small stomatal aperture/high stomatal resistance.

Plants require at least 14 minerals (essential elements) for growth (Taiz & Zeiger, 2010; Hawkesford *et al*., 2012). An essential element is defined as one that plants cannot complete their life cycle without (Arnon & Stout, 1939). According to their relative concentration in plant tissue, essential elements are classified as macronutrients, these are nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulfur (S) (plus silicon (Si) in a few plants) or micronutrients boron (B), chlorine (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) (Taiz & Zeiger, 2010; Hawkesford *et al.*, 2012). Mineral nutrients are essential for plant growth, and they are utilized in various biological functions. For example, N is a component of nucleic acids thus DNA and RNA, proteins thus enzymes, chlorophyll, some important co-enzymes, the phytohormones, auxin and cytokinins and a range of secondary metabolites (Andrews *et al*., 2013). Plant dry matter contains C and O both at about 40-45 %, H at about 6% and N at only 1-6% of total dry matter. All other elements make up a small proportion of plant dry weight (Epstein, 1972; Epstein, 1999; Hawkesford *et al*., 2012).

Plants can only show good growth within a certain range of temperature. C_3 temperate grass species including perennial ryegrass usually reach a maximum growth around 20 degrees (Parson & Chapman, 2000; Valentine & Mathew, 2002). Plants also need space to establish, grow and develop.

1.2 Factors which can limit plant growth

All factors necessary for plant growth (light, CO_2, H_2O, O_2, macro/micro-nutrients, suitable temperature, space) can limit plant growth if not in plentiful supply.

In New Zealand, pasture growth is primarily limited by N, P and S availability. Therefore, farmers commonly apply these elements as fertilizer to improve soil fertility and gain stable yield (White & Hodgson, 1999). Also, in certain areas like Canterbury, water availability severely limits growth. In Canterbury, irrigation is commonly used in the summer months.

1.3 NZ agriculture

Agriculture is a very important sector of the NZ economy. Half of the total exports and a large contribution to the gross domestic production (GDP) comes from agricultural products (Moot *et al*., 2009 & Statistics New Zealand, 2017). Farming systems, in particular grass dominant pastures, take up the most land in NZ. However, changing climate issues (like global warming and rainfall reduction) are threatening the agricultural industry. Therefore, drought has become an increasing focus for both plant breeders (Matthew *et al*., 2012) and meteorologists. Moreover, the NZ government has provided funding support for communities to set up irrigation to help farmers deal with the drought problems (Ministry of Primary Industry, 2017).

1.4 Perennial ryegrass

Perennial ryegrass (*Lolium perenne* L.) is the most commonly utilized grass species in New Zealand and in temperate pasture systems worldwide due to its high production, high feed quality (e.g. dry matter digestibility, metabolizable energy and crude protein - see Chapter 3) and easy management (Langer, 1990; Andrews *et al*., 2007). It is often mixed with white clover in pastures as white clover not only provides nitrogen for the grass via N_2 fixation, but it can also improve the pasture quality and milk production (Andrews *et al*., 2007). However, high yield of perennial ryegrass is not persistent during the summer drought period in NZ (Turner *et al*., 2006). Therefore, introduction of new germplasm and improvement of local perennial ryegrass has been critical work for breeders (Steward, 2006).

1.5 Objectives of study

In this study, growth and feed quality of three New Zealand cultivars, one cultivar Barberia, from Morocco and one Norwegian cultivar were compared under three water treatments. The five cultivars were Alto, Barberia, Bronsyn, Trojan and Norway (Ac 17183). The three water treatments were non-stress, mid-stress and high-stress.

In Chapter 2, NZ agriculture and pasture system were briefly introduced and water stress issues discussed. Previous and recent research on NZ cultivar performance under drought was reviewed and the five cultivars involved in this project described.

In Chapter 3, the five cultivars were compared under three water treatments and their dry matter production, leaf water content, leaf width, photosynthetic rate, stomatal conductance and feed quality determined. This was Experiment 1.

Chapter 4 described two small experiments. Firstly, plants under high-stress treatment in Experiment 1 were rehydrated and their subsequent DM production and feed quality determined. Secondly, a small ‘repeat’ experiment involving the three NZ cultivars and cultivar Barberia was used to check the findings under the high stress conditions of Experiment 1.

Chapter 5, the final chapter, discussed the results and findings of this project.

Chapter 2

Literature review

2.1 New Zealand agriculture and pasture system

The New Zealand agricultural industry plays a key role in the national economy and contributed $9,130 million to gross domestic production in 2013, $13,110 million in 2014, and $8,143 million in 2015 (Statistics New Zealand, 2017). Half of the total exports of NZ came from agricultural products (Moot *et al.,* 2009). Agricultural activities and land-use in general include pastoral farming, forestry, horticulture and arable crop production (Statistics New Zealand, 2012). Pasture farms (including sheep and beef, dairy, deer and horse farms) have the largest farm number and land area, especially sheep/beef farming with 5,865 farms and 4,354,784 hectares. Total pastoral land area reaches 12,086,333 hectares, over 80% of the total farm land (14,596,233 hectares). For most of the year, NZ livestock is primarily grass fed.

There are big sheep and beef farms in Canterbury, Otago, Manawatu-Wanganui, Southland and other hill and high country. Dairy farms are important in Waikato and Canterbury. Sheep and beef farmland reduced in size from 2002 to 2012, while dairy farms increased over that time in several regions, in particular, Canterbury from 345,076 hectares to 673,141 hectares, Otago from 150,363 hectares to 321,703 hectares and Southland from 218,717 hectares to 444,558 hectares (Statistics New Zealand, 2015). This conversion required a strong support of water supply especially in areas like Canterbury which have a period of summer drought. The government has provided large support for irrigation schemes in NZ (Ministry for Primary Industry, 2017).

2.2 Perennial ryegrass

Perennial ryegrass (*Lolium perenne*), family Poaceae, is one of the cool-season grasses which is widely sown in New Zealand and other temperate countries as a forage species (Langer, 1990). Long-lived perennial ryegrass has many tillers with hairless and flat leaves; the adventitious root is relatively shallow which causes vulnerable resistance to drought (Mayfield & Neilson, 1996). Besides water requirements, soil fertility and temperature can influence the growth of perennial ryegrass and a soil pH >4.8 is required. Optimum temperature for perennial ryegrass growth is around 18 degrees (White & Hodgson, 1999).

Perennial ryegrass is the dominant pasture in NZ because it suits the predominant climate (Turner *et al.,* 2006). In addition, its rapid establishment, the capability of being grazed or made into hay and silage, high nutrition and digestibility (75-80%) has made perennial ryegrass the number one choice for New Zealand pastures (Mackinnon *et al.,* 1988; Tonmukayakul, 2009). Perennial ryegrass is typically mixed with white clover (Harris, 1987; Andrews *et al*., 2007).

During the 1800s, British immigrants initially introduced perennial ryegrass germplasm with other pasture species (Stewart, 2006). Modern perennial ryegrass breeding in NZ started in the 1930s with a government plant research station in Palmerston North (Matthew *et al.,* 2012) and a variety seed certification system was established (Hunt & Easton, 1989). Shortly after this, seed companies like PGG Wrightson, and New Zealand Agriseeds Ltd started their own breeding programs. Current NZ perennial ryegrass varieties were mainly bred from the 'Hawkes Bay' and 'Mangere' ecotypes and introduced Spanish germplasm (He *et al.,* 2017a). Recently more extensive searches for novel endophyte perennial ryegrass arrangements and drought tolerant characteristics have also led to the breeding with ecotypes from other parts of the world such as Europe and Morocco (Pembleton *et al*., 2016a). An ecotype from Norway (Margot Forde Germplasm Centre Accession Number Ac 17183) was also shown to be potentially drought tolerant (Cyriac *et al*., 2017).

Although much research into perennial ryegrass drought tolerance characteristics has been carried out in NZ, real breeding efforts have been limited due to the outbreeding heterozygous nature of perennial ryegrass and the composite nature of many cultivars produced. Half-sib family selection is probably the most common current method of perennial ryegrass breeding (Nyugen & Sleper, 1983), though 'Aberdart' and 'Abermagic' varieties have been produced by continual reselection from within a population (Blackmore *et al*., 2016). Due to the difficult nature of drought traits, and the multiple definitions of what drought tolerance even means (Hussain, 2013), practical breeding for drought tolerance has been limited to introgression of perennial ryegrass from drought tolerant areas, e.g. the Spanish ecotypes, and selections from serendipitous weather events, or from drought prone areas, like Australia. In New Zealand, perennial ryegrass drought tolerance research has focused mainly on screening for drought tolerant differences (Cyriac *et al*., 2017, Matthew *et al*., 2017). Their recent work on variation of water use within and between perennial ryegrass populations and perennial ryegrass germplasm accessions responses to drought stress had different results. Matthew *et al*., (2017) found no significant differences of water use among perennial ryegrass populations but considerable variation within populations. Cyriac *et al*., (2017) observed the variation in drought responsiveness between accessions, but used small numbers and so may not differentiate between among and within population variation. Neither studies considered the phylogenetic distances between populations and may have missed genotypes with novel water use attributes. This study tried to address this issue by choosing lines from very distinct phylogenetic groups that lie within the *Lolium* genus and assessing their response to water stress.

In the current study, five cultivars were used as shown in Figure 2.1. Three of these cultivars are local cultivars Alto, Trojan and Bronsyn supplied by New Zealand Agriseeds Ltd, and the other two are cultivar Barberia from Morocco supplied by New Zealand Agriseeds Ltd and the line (Ac 17183) from Norway (Margot Forde Germplasm Centre, Palmerston North, New Zealand). These cultivars were chosen to represent distantly related germplasm from different origins in order to assess the diverse *Lolium* gene pool and are depicted on the neighbor-joining (NJ) dendrogram in Figure 2.1 (Pembleton *et al*., 2016a). Three local cultivars are from separate clusters of the perennial side of the dendrogram and extremely distant from Barberia a (*Lolium multiflorum*) genotype. The Norwegian ecotype is not represented on the dendrogram but reported to have good water use efficiency (WUE) attributes (Cyriac *et al.,* 2017).

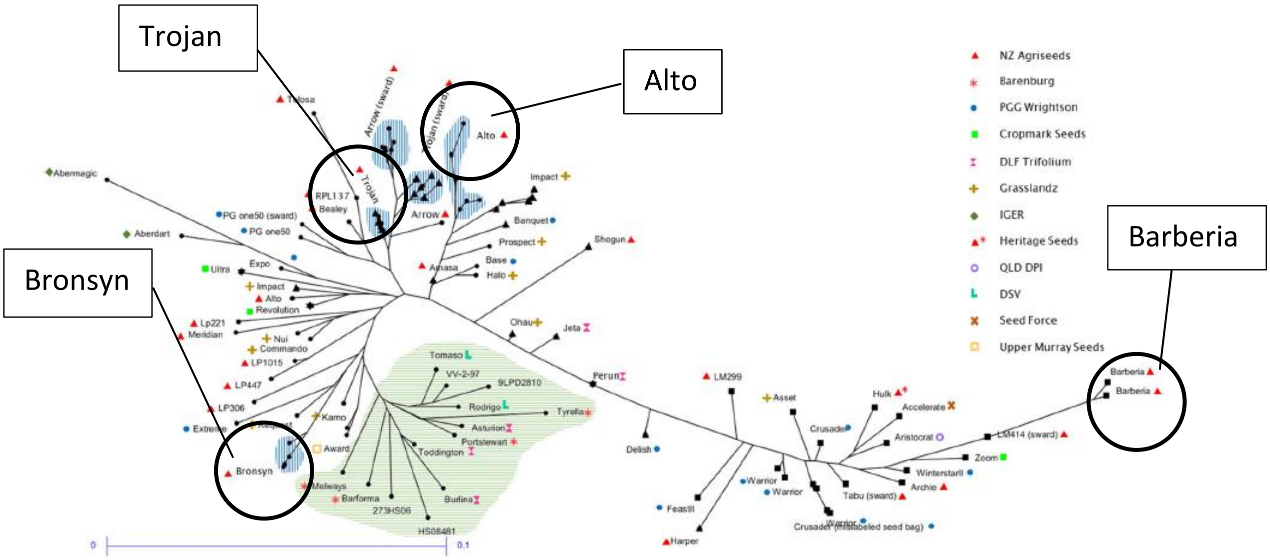

Figure 2.1 A neighbor-joining (NJ) dendrogram tree for cultivars of perennial ryegrass and location of four cultivars: Alto, Barberia, Bronsyn and Trojan, which were used in the current study.

2.2.1 Alto

Alto was bred from a cross between Bronsyn (Agriseeds NZ) and Impact (NZ Grasslands) (personal communication, Courtney Inch, Christchurch Agriseeds). Its parental mix is mostly from ecotype Mangere and some North-West Spanish ecotype material from Impact. Alto has an ear emergence date 14 days later than standard (Nui) and a persistent late heading date that is suited to most farming systems. It has fine leaves and dense tillers. Finer leaved cultivars on wet soils give soil protection against treading.

In the national forage variety trials, Alto had an average 14,000kg/ha annual yield based on the combined seasonal yields over all New Zealand trials Figure 2.2 (New Zealand Plant Breeding and Research Association (NZPBRA), 2016).

Illustration 2.1 Alto cultivar

2.2.2 Barberia

Barberia was selected in the Netherlands from a Moroccan ecotype (personal communication, Courtney Inch, Christchurch Agriseeds). It is very early heading and has summer dormancy with high winter yield. With adequate water, it has good summer performance and late summer growth after drought. It is highly palatable and grows like an annual grass but lasts like a long rotation grass, persisting 4-5 years. In Heritage Seed, Australia research (2007), Barberia exhibited a higher winter and summer yield than other cultivars.

Illustration 2.2 Barberia cultivar

2.2.3 Bronsyn

Bronsyn is a diploid perennial ryegrass selected from New Zealand Mangere ecotype material for yield and persistence under Northern North Island conditions. It shows a standard heading date and has outstanding crown rust resistance, persistent summer and autumn production and good winter growth in mild winters, making it also popular in France, Source: http://www.barenbrug.biz/forage/products/bronsyn.htm. Bronsyn showed a high plant pulling score in six trials from 2003-2011 comparing 18 ryegrass cultivars (Kerr *et al*., 2012), suggesting either weak root systems or strong leaves. However, the dry matter (DM) yield results were varied depending upon the endophyte used. For example, Bronsyn with NEA6 endophyte had higher DM than Bronsyn with SE and AR1 endophyte (Kerr *et al.,* 2012).

Illustration 2.3 Bronsyn cultivar

2.2.4 Trojan

Trojan is from a North-West Spanish ecotype material with high year-round yield and a late heading date (+16 days) in comparison with ordinary cultivars, providing high feed quality in late spring and summer (Agriseeds, 2017). It contains NEA2 endophytes, which give good animal health and insect control (Kerr *et al.,* 2012).

Illustration 2.4 Trojan cultivar

In the NZPBRA trials, Trojan showed a top yield performance over all seasons compared to other cultivars in Figure 2.2 (NZPBRA, 2016).

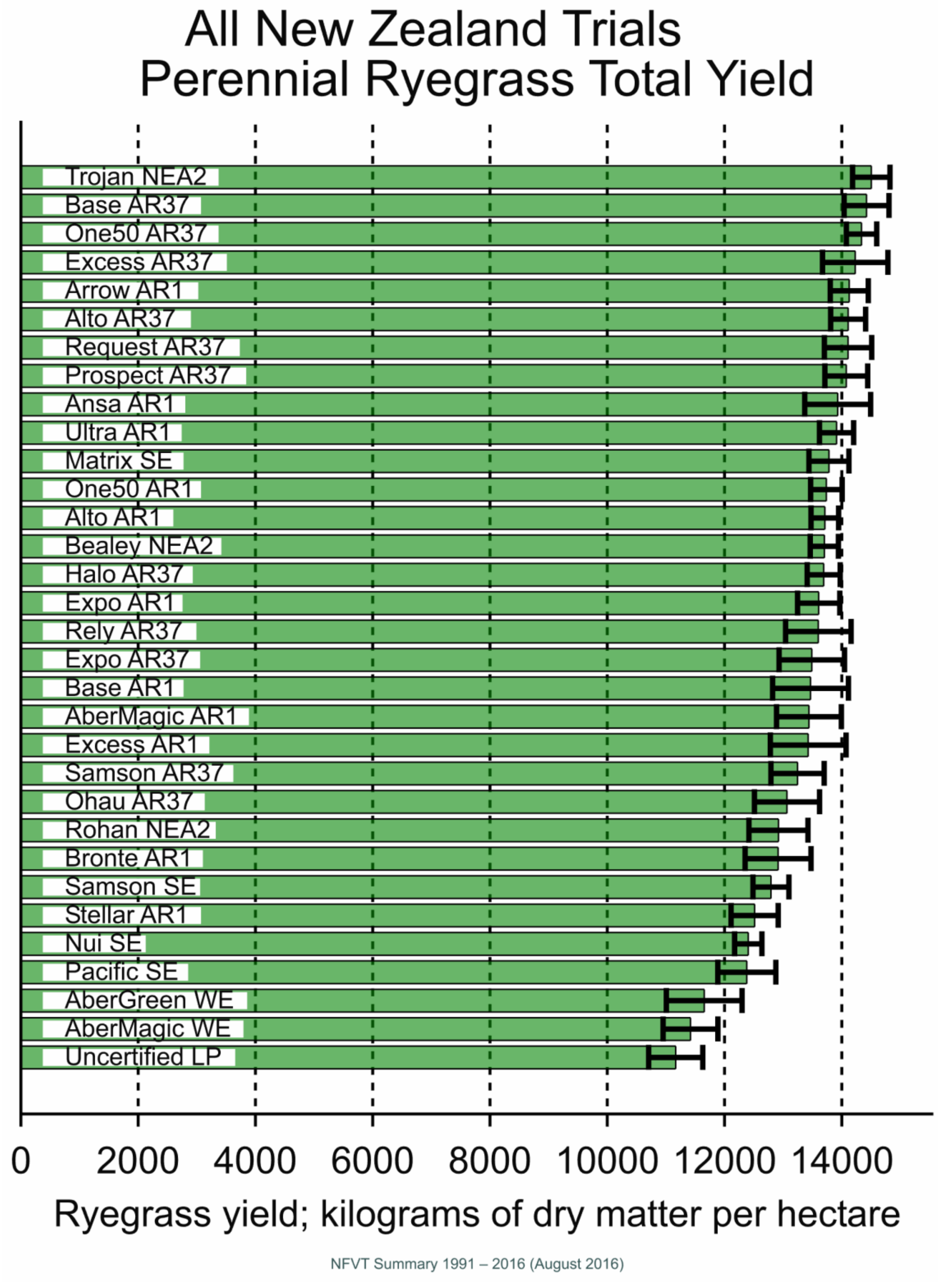

Figure 2.2 All New Zealand trials perennial ryegrass total yield.

2.2.5 Norway

The Norwegian cultivar (Ac 17183), 'cv Norway', is an increase of the Ac 16719 collection from Norway held at the Margot Forde Germplasm Centre, Palmerston North, New Zealand, that was originally collected in Bryne, South-West Norway and maintained by the Western PI station. In 1991, this accession was donated to the National Plant Germplasm System (NPGS) in Wales (Samuel, 2016). The climate of South-West Norway is different from NZ. The driest months start from April to July and the hottest period is June and July with maximum temperature about 16 degrees. Summer temperature is lower than in NZ. Cultivar Norway is a diploid perennial ryegrass with medium tillers and small leaves.

Previous research on this accession showed it had a greater shoot biomass than the NZ cultivar Impact and the other cultivars after three drought cycles (Cyriac *et al.,* 2017). This could have resulted from its early morphology and physiology responses to drought (Samuel, 2016). It was reported that the Norway cultivar could have reduced leaf extension rate (LER) more than the other cultivars which preserved resources in early drought cycles. No osmotic adjustment or changes in the chlorophyll concentration were found in cv Norway under drought (Samuel, 2016).

Illustration 2.5 Norway (Ac 17183) cultivar

2.3 Feed quality

Plant feed quality or nutritional values is closely related with animal health (Ulyatt, 1981; da Silva *et al*., 1987; Stone, 1994) and as such is another trait that breeders have to consider. Near Infrared Spectroscopy (NIR) is a cheap, simple and relatively rapid measurement method for feed quality testing (Pembleton *et al*., 2016b). The feed quality (dry matter digestibility, crude protein and metabolizable energy) test in Chapter 3 used this approach. Forage is ranged from poor quality (high fibre, low digestible, low intake) to high quality (high protein, low fibre, high metabolism energy) (Rattray *et al*., 2007).

Dry matter digestibility (DMD) can be measured by an in vitro technique known as in vitro digestible dry matter (CDDM) as well (Wilson, 1983), which has been cited as the single best indicator of nutritional value (Casler, 2001; Wilkins & Humphreys, 2003; Andrews *et al*., 2007), since it influences both grass intake and energy availability (Wilkins & Humphreys 2003). Casler & Vogel (1999) reported an average 3·2% increase in live weight gain by beef cattle per 1% increase in grass digestibility.

Crude protein (CP) in grass is a major source of nitrogen for protein synthesis by microbes in the rumen. In grazed swards, perennial ryegrass CP concentration is relatively high among forages and is invariably above the threshold of 80 g CP/kg DM required for rumen function (Coleman &Moore, 2003).

Metabolizable energy (ME) is the value of animal maintenance and production. It is used to indicate the available energy, the economic value, quality of supplements and forages (Waghorn, 2007). The crops with higher ME value can provide more digestible energy for animals.

2.4 Perennial ryegrass and water stress

Perennial ryegrass has several desirable agronomic characteristics, but these are not expressed optimally under drought condition (Turner *et al*., 2006). High potential yield can only be possible when water and other factors are unrestricted. Matthew *et al.,*(2012) indicated that potential yield could reach 15.8 t DM/ha in Southland with 1208 mm annual rainfall and 19.9 t DM/ha in Waikato with 1332 mm annual rainfall. However, Southland only had 1134 mm average annual rainfall (2001-2010) and Waikato had 1121 mm which is over 200 mm water deficiency of annual rainfall. Economically, the severe drought in the 2012/2013 summer caused an estimated $1.3 billion economic impact (Ministry for Primary Industries, 2013). Meanwhile, drought conditions are likely to become more frequent and severe due to climate changes (Hollis, 2014). To deal with this possible coming hazard, the National Institute of Water and Atmospheric Research (NIWA, 2016) has provided a series of drought indicators for farmers and researchers to understand the current and historical changes of drought conditions around the country. For example, the Standardized Precipitation Index (SPI) is related to the rainfall situation. The Soil Moisture Deficit (SMD) can show the soil moisture which is closely related to pasture growth. Recently (2017 March 24th), the NIWA (2017) launched a new tool called The New Zealand Drought Index (NZDI) that combines all indices into one overall index that can simply display the dryness with a color-coded map. From the website video, NZDI shows the drought condition between 1st January and 21st March. It clearly records the changes of drought area around the country. For example, the North Island east coast, had a constant and even severe dry situation between January and March. Canterbury and Southland recorded a large area which was dry or very dry between February and March. A short time of severe drought occurred in the southern part of the South Island as well over this period (https://www.niwa.co.nz/news/niwa-launches-new-zealand-drought-index-a-one-stop-drought-monitor).

Where possible and if economically viable, irrigation is the common solution to drought in NZ. NZ began large-scale irrigation in the late 19th century supported by the government but this support stopped in 1988 (Irrigation New Zealand, 2017). The Canterbury area had a quick development of irrigation (Figure 2.3), irrigated area increased from less than 300,000 ha in 2002 to more than 500,000 ha in 2015 (Environment Canterbury, 2016). In 2017, government started to again provide funding support for communities to set up irrigation systems (Ministry for Primary Industry, 2017).

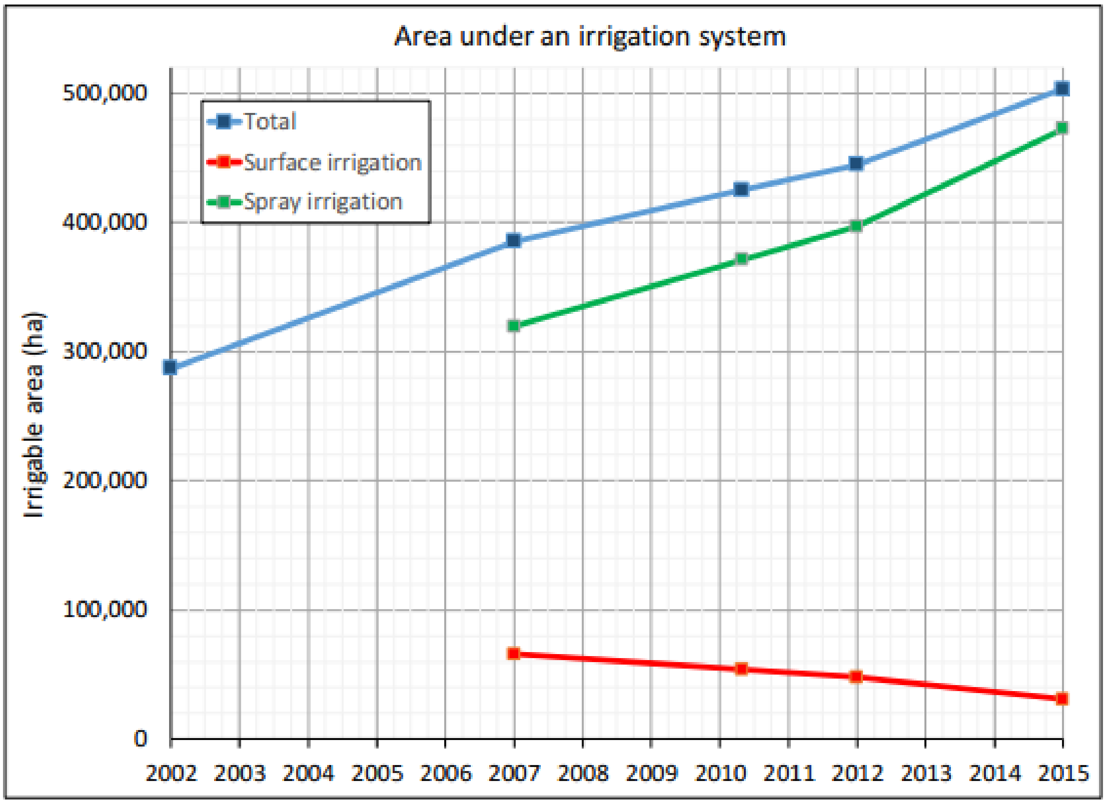

Figure 2.3 Changes in irrigated area in Canterbury over time.

However, for many parts of NZ, especially hill country regions, irrigation is not possible. For these regions, evaluation and selection of cultivars with good drought tolerance is a more prospective method than irrigation. Therefore, it is vital for breeders to understand the current cultivars' performance under drought condition and select out more potential genotypes to improve the seeds quality.

This Masters project compared growth and feed quality of three New Zealand cultivars (Alto, Bronsyn and Trojan), one cultivar Barberia, from Morroco and cv 'Norway' from Norway under three water treatments: non-stress (field capacity, FC), mid-stress (50% FC) and high-stress (30% FC).

Chapter 3

Effect of three water treatments on growth and feed quality of five perennial ryegrass cultivars

3.1 Introduction

Water moves from the soil through the plant to the atmosphere via the soil-plant-atmosphere continuum (McLaren & Cameron, 1998; Blum, 2011), following a water potential gradient. Water potential is a measure of the ability of a substance that contains or could contain water to attract water. The unit of water potential is the unit of pressure MegaPascall (MPa) with pure water the reference point at 0. Water content is commonly described in a gravimetric way as mm water per m soil. When soil contains all the capillary water it can retain against gravity, it is said to be wet to field capacity (McLaren & Cameron, 1998). The minimal soil water by plant to avoid wilting is called the permanent wilting point, and is approximately 1.5 MPa, but is dependent on crop. The available water is the difference in the amount of soil water between field capacity and permanent wilting point.

Perennial ryegrass (*Lolium perenne)* is the most common pasture species grown in New Zealand due to its high production, high feed quality and easy management (Harris, 1987; Langer, 1990; Andrews *et al*., 2007). However, high yield of perennial ryegrass is not persistent during the summer drought period in NZ (White & Hodgson, 1999; Turner *et al*., 2006). The Ministry for Primary Industries (2013) reported that NZ economy lost about $1.3 billion due to drought. Therefore, the development of a drought tolerant perennial ryegrass cultivar has become a new focus for the NZ forage industry (Matthew *et al*., 2012). However, there is little reference to drought performance for current commercial lines and potential drought tolerant germplasm from overseas.

Water deficit can cause a series of morphological and physiological effects in plants. Decreased leaf area is shown at the early stage of drought due to decreased leaf expansion, leaf rolling and leaf wilting. Also, photosynthetic rate per unit area is reduced due to stomatal closure (Lawlor & Cornic, 2002). These changes lead to a decrease in overall photosynthesis at plant and pasture level, showing as production decline.

In this chapter, the five cultivars Alto, Barberia, Bronsyn, Trojan and Norway were compared under three water treatments (100 % field capacity (FC) as non-stress, 50% FC as mid-stress, 30% FC as high-stress) and their shoot dry matter production, leaf water content, photosynthetic rate, stomatal conductance and feed quality determined. This was Experiment 1.

3.2 Material and methods

Experiment 1 was carried out in a greenhouse at company Agriseeds, Canterbury (43°27’ S, 172°11’ E) from April 22nd, 2016 to mid-March 2017.

Establishment and water treatments

Three commercial lines of Agriseeds were used (Alto, Trojan and Bronsyn). Alto and Bronsyn were collected from the company trial field along with Barberia plants. Trojan was brought from the trial field in New Zealand North Island. Norway (Ac 17183) was germinated from seeds (April 22nd). Four plants of each cultivar were selected and moved to pots (June 3rd). All plants were grown for two months prior to further selection for Experiment 1.

Three to five tillers of each plant were transplanted into 4L experimental pots after the irrigation system and pots were set up. In the first month, all the plants were maintained at field capacity to let them establish in the pots.

Plants were grown in 4 Litre pots containing, a short-term (3 month) potting mix obtained from company Intelligro, Rolleston NZ. The five cultivars (Alto, Barberia, Bronsyn, Trojan and Norway) were tested. The experiment was a randomised block design with 3 water treatments (100 % FC as non-stress, 50% FC as mid-stress and 30% FC as high-stress) x five cultivars x four replicates with each replicate the mean of 3 pots. Therefore, there was 3 x 5 x 4 x 3 = 180 pots in total (Illustration 3.1). Initially, soil water potential was determined, using nine water potential sensors, three for each water treatment. However, values from these water potential sensors were very variable and unstable, therefore, in order to achieve the three water treatments, pots were weighed every 2-3 days and water levels adjusted manually to maintain the three water treatments (Illustration 3.2). The field capacity of the potting mix was determined as described in Liu *et al*., (2014). Soil was added to 15-cm-height x 9-cm-diameter pots with a layer of cheese cloth at their base and was kept almost immersed in a beaker of water for 36 h. The pots were then removed from the water covered with plastic wrap and left to drain for 36 hours. After this, the soil was weighed, dried at 105 degrees for 24 hours and reweighed, and g H_2O kg^{-1} fresh weight soil was determined.

Illustration 3.1 Glasshouse in Agriseeds and layout of Experiment 1.

Experimental conditions

Experiment 1 was conducted in a greenhouse room at Agriseeds company. The greenhouse room temperature generally ranged between 19 and 30℃, but on occasion reached 40℃ for around 2 hours in hot middays. The photoperiod was 16 hours/day with natural light supplemented by halogen lights as required.

Illustration 3.2 Water treatments control and unification via weight by using scales and weighing pots every second day.

The three water treatment levels were reached by the second harvest, then maintained until the end of the experiment.

Fresh weight and Dry matter production and leaf water content

The grasses were cut 4-5 cm above the ground (Davis *et al*., 1993) with shears and fresh weight recorded immediately. The plant material was then dried at 70℃ for at least 72 hours. Then dry matter (DM g/pot) and leaf water content determined.

Leaf water content (LWC) = (Fresh weight – Dry weight)/Fresh weight %

Four harvests were carried out between 10th November 2016 and 4th January 2017, every second Thursday.

Photosynthetic rate and stomatal conductance

Forty-five pots (3 replicates x 5 cultivars x 3 water treatments) were selected out and moved to Lincoln University glasshouse one week before testing photosynthetic rate to let plants adapt to the new environment. Photosynthetic rate was tested from 11:00am to 2pm on 10th March 2017 with a LI-COR LI-6400 XT portable infra-red gas analyser unit (LI-COR Biosciences, Inc. Lincoln, Nebraska, U.S.A.) (Illustration 3.3).

The LI-6400 is primarily designed for broad leaves, with a measurement area of 6cm² (2cm x 3cm). Every time a leaf is clamped by the LI-6400 and tested, the output data assumes a measurement area of 6cm². Because the grass leaves were less than 2cm wide, the actual leaf width and subsequent leaf measurement area had to be determined, to account for the reduction in area (and gas exchange), compared to the default setting (6cm²). Leaf width was determined at the centre of each leaf with an electronic digital caliper and multiplied by the LI-6400 chamber length (3cm) to obtain the leaf measurement area. The trial plant leaves were initially measured with the default leaf area, then the data was later recomputed on a LI-6400 generated Microsoft Excel sheet taking the measured leaf area into account. The output data consisted of photosynthetic rate, stomatal conductance and a number of other variables which are not presented in the thesis.

Illustration 3.3 Photosynthetic rate and stomatal conductance measurements were carried out in Lincoln University glasshouse with a LI-COR LI-6400 XT portable infra-red gas analyser unit (LI-COR Biosciences, Inc. Lincoln, Nebraska, U.S.A.).

Feed quality test

The third and fourth harvests were selected for the feed quality tests. Dry material samples were ground into powder through a 1 mm steel sieve (model: Ultra Centrifugal Mill ZM 100, Retch GmbH, Haan, Germany). Near Infrared Spectroscopy (NIR) was used to do the feed quality tests (dry matter digestibility, crude protein and metabolizable energy) at Lincoln University between March 22nd and March 28th, 2017 (Machine: FOSS, NIR Systems 5000, Maryland, USA) (Illustration 3.4).

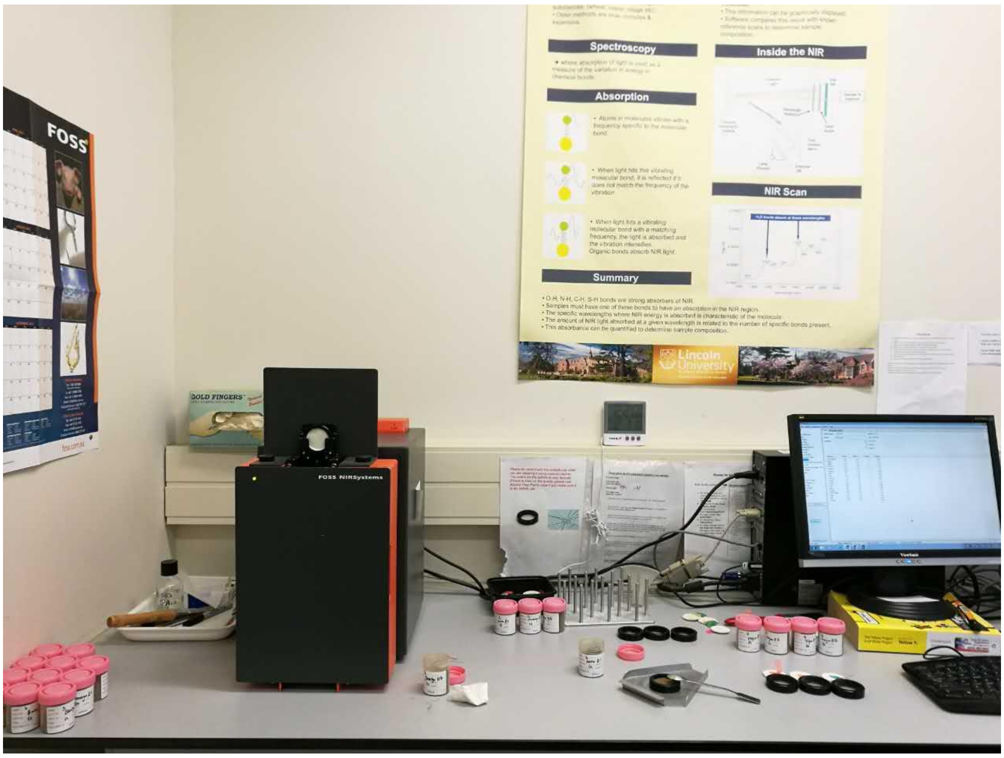

Illustration 3.4 The FOSS, NIR Systems 5000 used in the feed quality tests.

Statistical analyses

A one way or two way analysis of variance (Anova) was carried out on all data as appropriate with cultivar and water treatment as fixed variables. All statistical analyses were performed using Genstat statistical analysis package (Version 17, VSN International Ltd).

3.3 Results

3.3.1 Dry matter production

There was a significant effect of water stress treatment, time, and an interaction between treatment and time, on shoot DM (g/pot) (P< 0.001 in all cases; Figure 3.1). However, there was no cultivar effect on shoot DM production regardless of water treatment. For all cultivars at Harvest 1, DM under all 3 water treatments was similar (Figure 3.1). By Harvest 2, shoot growth in the high-stress plants was lower than the other two treatments, and by Harvest 3, the three treatments were very distinct for all cultivars (Figure 3.1). At the fourth harvest, gaps between mid-stress and high-stress still remained but the non-stress plants of all cultivars dropped to similar levels as the mid-stress treatment.

Generally, all five cultivars had a similar shoot response to the three water treatments, and there was no significant difference between cultivars (P = 0.089: Figure 3.1). However, Barberia appeared to have a greater DM than all other cultivars which were similar under non-stress conditions but NB not significant (Figure 3.1), and therefore showed the highest difference in DM between non-stress (4.6 g/pot) and mid-stress (1.9 g/pot) plants.

The accumulated shoot DM (g) over the four harvests was not affected by cultivar (P = 0.206). However, there was a clear effect of water stress treatment (P < 0.001), with the non-stressed plants producing almost twice DM of the highly stressed plants (Table 3.1).

When only the last two harvests were considered (Table 3.2), the non-stressed plants produced around four times the DM of the highly stressed plants. Mid-stress plants had almost three times the DM of the high-stress plants (Table 3.2).

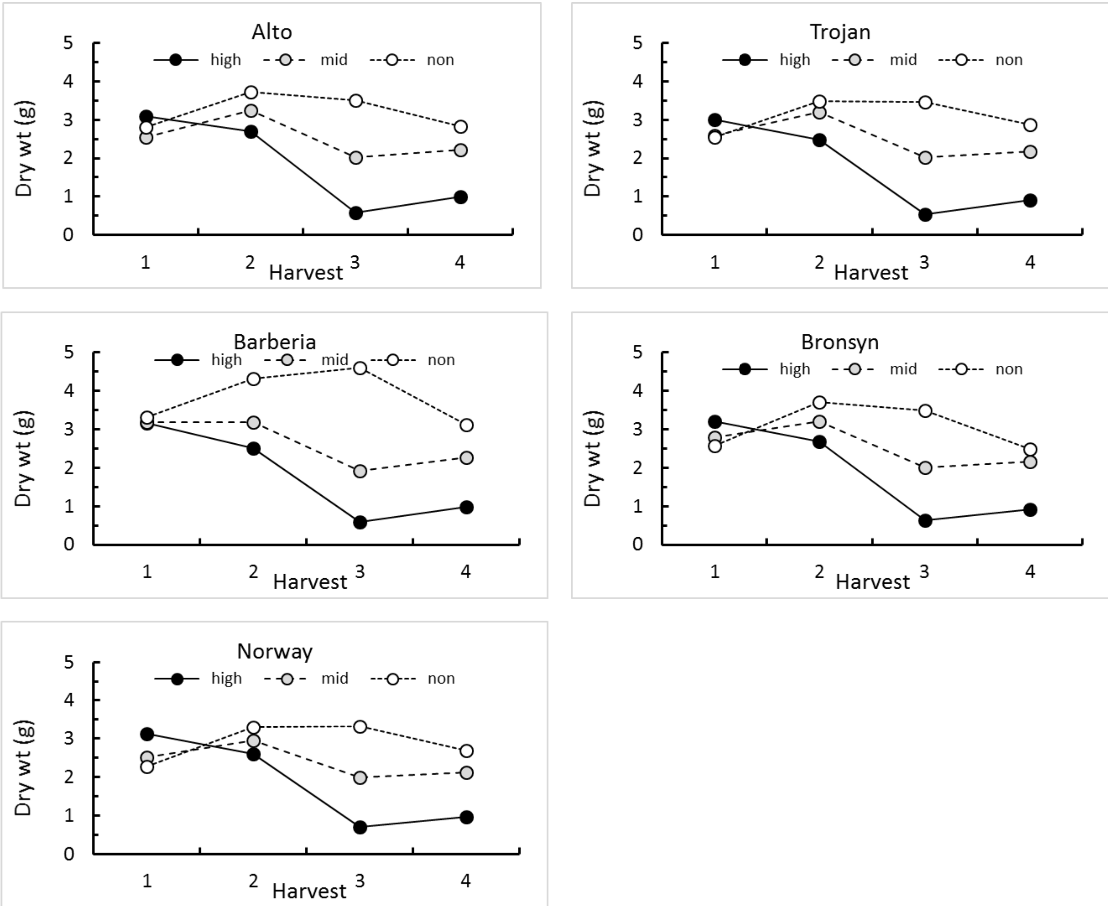

Figure 3.1. Leaf dry matter (mean: DM g/pot) production of five perennial ryegrass cultivars over four harvests (two weeks apart), with three water regimes (non-stress, mid-stress and high-stress). See Appendix Table 1 for standard deviation.

Table 3.1. Total shoot dry matter (mean: DM (g/pot)) production of five perennial ryegrass cultivars obtained over four harvests, with three water regimes (non-stress, mid-stress and high-stress).

	Stress		
CV	high	mid	non
Alto	7.40	10.17	12.92
Barberia	7.39	10.37	15.44
Bronsyn	7.14	10.45	12.31
Norway	7.12	9.73	11.59
Trojan	7.15	9.99	12.20

Table 3.2 Total shoot dry matter (mean: DM (g/pot)) production of five perennial ryegrass cultivars obtained over last two harvests (Harvest 3 and Harvest 4), with three water regimes (non-stress, mid-stress and high-stress).

	Stress		
CV	high	mid	non
Alto	1.58	4.25	6.33
Barberia	1.59	4.19	7.73
Bronsyn	1.55	4.15	5.99
Norway	1.68	4.13	6.02
Trojan	1.45	4.20	6.35

3.3.2 Leaf water content (LWC)

There was a significant effect of time and cultivar, and an interaction between time and water treatments, on leaf water content % ($P<0.001$ in all cases; Figure 3.2). At Harvest 1, as for DM production, LWC under all treatments was similar. At later harvests, LWC in the high-stress plants experienced a continuous decrease and was lower than plants in the other two treatments until Harvest 4. Mid-stress and non-stress plants also showed a decline of their shoot LWC but this was not as severe as that seen in the high-stress treatment (Figure 3.2).

Among the five cultivars, Trojan had the lowest LWC at the Harvests 1, 2 and 3. At the fourth harvest, Norway became the lowest LWC cultivar (Figure 3.2). Norway was the most severely affected cultivar in terms of having the lowest LWC in the high-stress treatment by Harvest 4 (Fig 3.2)

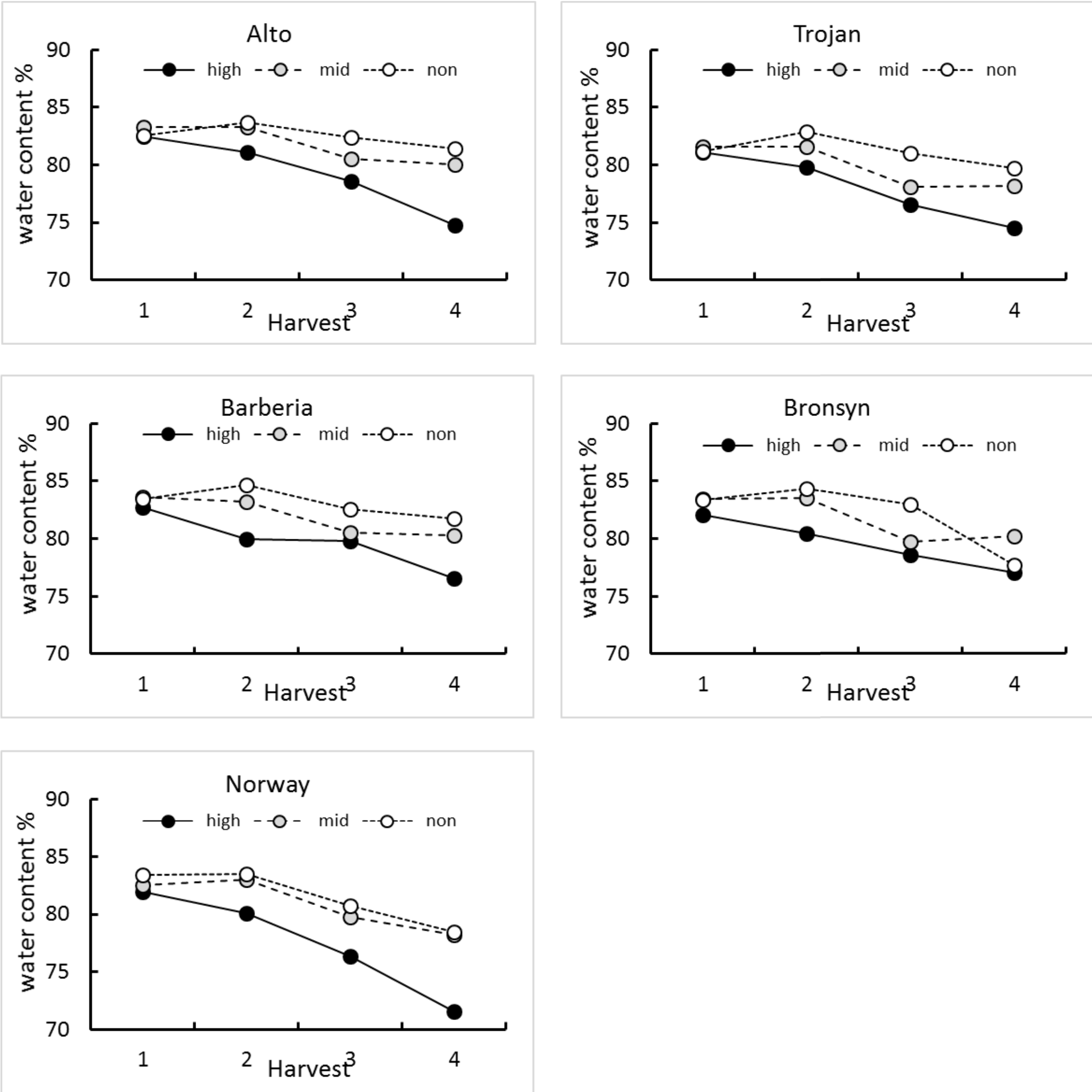

Figure 3.2. Leaf water content (mean: LWC %) of five perennial ryegrass cultivars over four harvests (two weeks apart), with three water regimes (non-stress, mid-stress and high-stress). See Appendix Table 2 for standard deviation.

3.3.3 Leaf dimensions and photosynthesis

Leaf width

There was a significant difference in leaf width of plants under the three water treatments ($P<0.001$). Over all the cultivars, water stress reduced the leaf width from 3.314mm under non-stress to 2.917mm under mid-stress and 2.561mm under high-stress. There were significant differences in leaf width among the five cultivars ($P<0.001$). Cultivar Barberia had the widest leaf under all three water treatments, but especially under non-stress condition (Figure 3.3)

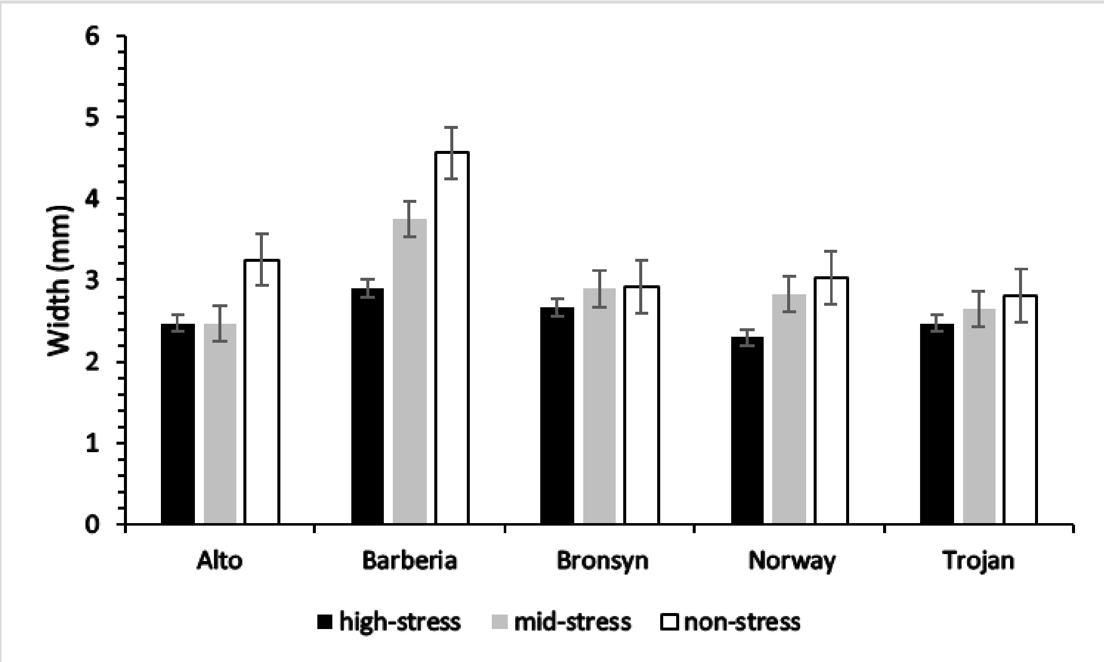

Figure 3.3 Leaf width (mm) of five perennial ryegrass cultivars under 3 water treatments (high-stress, mid-stress and non-stress).

Photosynthetic rate and conductivity

There was no significant difference among cultivars with regard to the rate of photosynthesis (PR; P = 0.172), but there was a highly significant difference among the three water stress treatments (P < 0.001). Over all cultivars, PR was highest in the non-stressed plants (14.55 µmol CO_2 m^{-2} s^{-1}) compared with the mid-stressed (11.92 µmol CO_2 m^{-2} s^{-1}) and high-stressed plants (10.43 µmol CO_2 m^{-2} s^{-1}).

Similarly, there was no significant difference among cultivars with regard to stomatal conductance (P = 0.348), but again there was a highly significant difference among the three water stress treatments (P < 0.001). Overall the cultivars, conductance was highest in the non-stressed plants (0.258 mol H_2O m^{-2} s^{-1}) compared with the mid-stressed (0.129 mol H_2O m^{-2} s^{-1}) and high-stressed plants (0.093 mol H_2O m^{-2} s^{-1}).

The trends for PR and conductance among the three stress treatments resulted in a strong relationship between PR and conductivity over all the cultivars (Fig 3.4). The high values for PR and conductance in the non-stress treatment made these plants quite distinct from the mid- and high-stressed plants in terms of these variables (Fig 3.4).

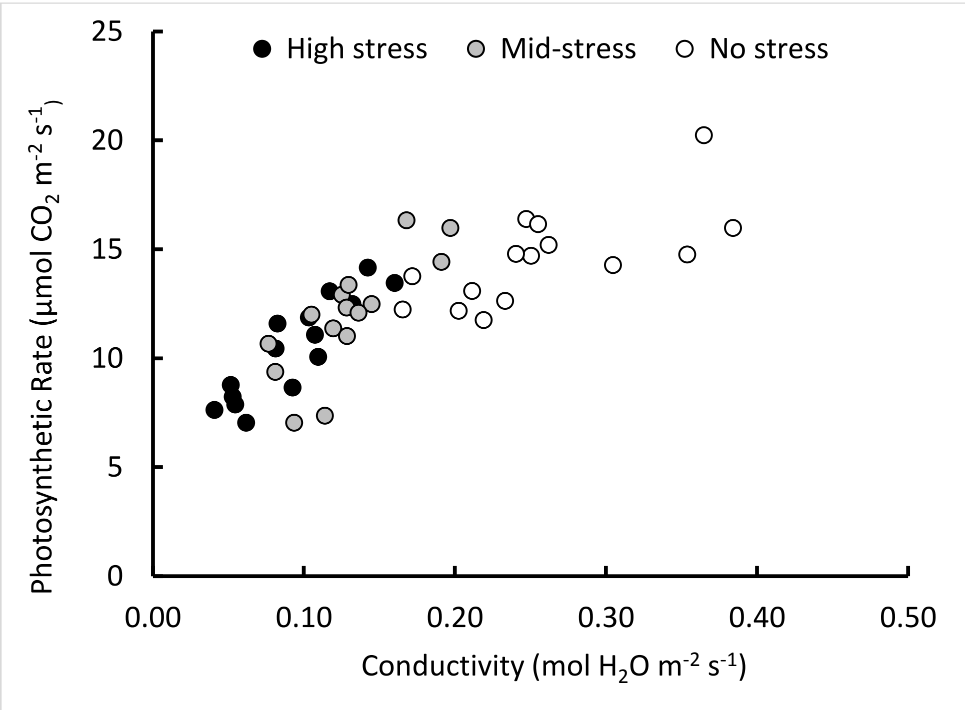

Figure 3.4 The correlation between leaf photosynthetic rate and leaf stomatal conductivity for five perennial ryegrass cultivars under three water treatments (Experiment 1).

3.3.4 Feed quality

Table 3.3 Cultivar comparison in dry matter digestibility (DMD), crude protein (CP) and metabolizable energy (ME) under the non-stress, mid-stress and high-stress water treatment for 3rd harvest by NIR.

		Cultivar				
	Water-stress	Alto	Barberia	Bronsyn	Trojan	Norway
	Non	71.84	68.37	70.45	72.02	73.51
DMD (%)	Mid	71.21	72.42	70.15	73.69	74.78
	High	72.54	73.58	71.79	74.00	75.08
	Non	18.8	17.33	17.98	19.05	20.69
CP (%)	Mid	25.07	26.52	25.22	23.44	24.73
	High	24.06	25.75	23.35	22.29	24.01
	Non	10.57	10.15	10.39	10.63	10.99
ME(MJ/Kg)	Mid	10.52	10.78	10.40	10.99	11.10
	High	10.86	11.07	10.77	11.13	11.33

At the third harvest, the water treatments had a significant effect on CP and ME ($P<0.001$), and DMD ($P=0.001$). DMD and ME showed significant differences among cultivars ($P<0.001$). There were no significant interactions between cultivars and water treatments with regard to all three variables (Table 3.3).

DMD was higher under drought conditions for all cultivars. On average, Norway (74.46 %) and Trojan (73.24 %) had higher DMD than the other three cultivars (Table, 3.3). Samples from mid-stress treatment had the highest CP. For ME, high-stress plants were slightly higher than the mid-stress and then non-stress. Similar to DMD, ME was slightly higher in Norway and Trojan than the other three cultivars (Table 3.3).

Table 3.4. Cultivar comparison in dry matter digestibility (DMD), crude protein (CP) and metabolizable energy (ME) under the non-stress, mid-stress and high-stress water treatment for 4th harvest by NIRS.

		Cultivar				
	Water-stress	Alto	Barberia	Bronsyn	Trojan	Norway
DMD/%	Non	72.06	70.14	70.37	72.28	73.45
	Mid	72.38	73.97	70.67	75.06	75.71
	High	73.94	74.57	72.37	75.45	76.65
CP/%	Non	18.03	17.55	17.92	18.78	19.73
	Mid	23.19	24.79	23.12	22.55	23.02
	High	24.83	25.84	23.72	23.01	24.92
ME(MJ/Kg)	Non	10.59	10.40	10.45	10.72	11.00
	Mid	10.71	11.06	10.50	11.24	11.36
	High	11.10	11.25	10.82	11.43	11.60

At Harvest 4, the results for DMD, CP and ME were broadly similar to those obtained at Harvest 3 (Table 3.3 & Table 3.4). The water treatments had significant effects on DMD, CP and ME ($P<0.001$). DMD and ME also showed significant difference among cultivars, but no interaction between cultivars and water treatments occurred for all three variables.

DMD was again higher under drought conditions, high-stress had the highest DMD. Norway (75.27%) and Trojan (74.26%) were higher than other three cultivars on DMD on average (Table 3.4). For ME, high-stress was slightly higher than mid-stress and non-stress. However, unlike Harvest 3, samples from high-stress plants now had the highest CP for all cultivars.

3.4 Discussion

Drought is a major cause of decreased production of perennial ryegrass pastures in the summer in important agricultural areas such as the Canterbury plains in New Zealand (Ministry for Primary Industries, 2013). Because of this, the development of a drought tolerant perennial ryegrass cultivar has become a new focus for the NZ forage industry (Matthew *et al*., 2012). However, there is little reference to drought performance for current commercial lines and potential drought tolerant germplasm from overseas.

In Experiment 1, the five cultivars Alto, Barberia, Bronsyn, Trojan and Norway were grown under three water treatments (100 % field capacity as non-stress, 50% field capacity as mid-stress, 30% field capacity as high-stress) and their shoot dry matter (DM) production, leaf water content (LWC), leaf width, photosynthetic rate, stomatal conductance and feed quality compared.

All five cultivars showed similar decreases in shoot DM (g/pot) in response to the increased water stress: there was no significant difference between cultivars. The differences between water treatments were more pronounced in the last two harvests since the water treatments became fully distinguished after the third harvest. Over the four harvests, plants under high-stress treatment had an average 7.24 g/pot with 10.14 g/pot under mid-stress and 12.89 g/pot under non-stress. When the lasts 2 harvests were combined, the non-stress block had an average of 6.48 g/pot, which was four times the average DM of the high-stress blocks (1.57 g/pot). This emphasises the crucial function of water to all cultivars of perennial ryegrass and these results are similar to these related in previous studies (Cui *et al*., 2015; He *et al*., 2017b; Matthew *et al*., 2017). Matthew *et al.,* (2012) concluded that it was unrealistic to hope to produce a cultivar of perennial ryegrass which maintains high production during the summer drought period in NZ. However, Cyriac *et al*., (2017) observed the variation in drought responsiveness between cultivars.

Water deficit can cause a series of morphological and physiological effects to plants. Decreased leaf area is shown at the early stage of drought due to decreased leaf turgor and hence decreased leaf expansion due to leaf rolling and leaf wilting. Also, photosynthetic rate per unit area is reduced due to stomatal closure which can be measured as decreased stomatal conductance (Lawlor & Cornic, 2002). These changes lead to a decrease in overall photosynthesis at plant and pasture level, which results in a decline in production. These effects were shown here. Specifically, for all cultivars, leaf water content decreased with increased water stress (O'Neill *et al*., 2006; Jansen *et al.*,2009; Luo *et al*., 2011; Yu *et al.*, 2013), although, there were small significant differences across cultivars. This decreased leaf water content was linked to a decrease in leaf width with increased water stress for all cultivars. This would result in reduced area for light absorption and photosynthesis. Also, leaf length and leaf number are likely to have been reduced but these measurements were not taken. These effects would result in further reductions in leaf area for light absorption and photosynthesis. Photosynthetic rate per unit area was determined and shown to decrease to a similar extent with increased water stress for all cultivars. Decreased photosynthetic rate is at least in part likely to have been related to reduced stomatal conductance which indicates smaller stomatal aperture. There was a strong positive correlation between photosynthetic rate per unit leaf area and stomatal conductance across all cultivars and water treatments. These results are similar to those of Hussain (2013), when they compared photosynthetic rate and stomatal conductance among four perennial ryegrass cultivars, they also concluded that the four cultivars did not significantly differ but water treatments had significant effects on both photosynthetic rate and stomatal conductance.

Water stress affected feed quality and this was dependent on cultivars. Across the two harvests, DMD and ME were greater under the high-stress conditions and also greater for Norway and Trojan than other three cultivars. Further chemical analysis would be required to determine the reasons for these differences. The finding of increased DMD, CP and ME under water stress was consistent with the study of Abraha *et al.,* (2015), which compared annual ryegrass feed quality under different water levels, and found increasing digestibility, ME and CP values under low water levels. However, the decrease in DM production under water stress is so great that any slight increase in feed quality would make little difference to animal production.

Chapter 4

Plant recovery after drought and a small 'repeat' experiment

4.1 Introduction

Decreased production of perennial ryegrass under drought condition can cause large production losses to the pasture industry (White & Hodgson, 1999 & Turner *et al*., 2006). Severe drought can cost NZ about $1.3 billion (Ministry for Primary Industries, 2013). Therefore, the development of a drought tolerant perennial ryegrass cultivar has become a new focus for the NZ forage industry (Matthew *et al*., 2012). However, there is little reference to drought performance for current commercial lines and potential drought tolerant germplasm from overseas.

In Chapter 3, five perennial ryegrass cultivars (Alto, Barberia, Bronsyn, Trojan and Norway) were grown under three water levels (100 % field capacity (FC) as non-stress, 50% FC as mid-stress, 30% FC as high-stress) and their DM production, leaf water content, leaf width, stomatal conductance and photosynthetic rates, and feed quality (dry matter digestibility, crude protein and metabolizable energy) measured. The water treatments had significant effects on DM production, LWC, leaf width, photosynthetic rate and stomatal conductance and feed quality. Specifically, water stress reduced DM production, LWC, leaf width, photosynthetic rate and stomatal conductance showed similar responses to water stress but increased dry matter digestibility, crude protein and metabolizable energy. Generally, the cultivars showed similar responses to water stress.

This chapter described two experiments. Firstly, plants under high-stress treatment in Experiment 1 were rehydrated and their subsequent DM production and feed quality (dry matter digestibility, crude protein and metabolizable energy) determined. Secondly, a small 'repeat' experiment (Experiment 2) involving Alto, Bronsyn, Trojan and Barberia grown under high-stress treatment was carried out dry matter production, leaf water content and feed quality (dry matter digestibility, crude protein and metabolizable energy) to check the findings of Experiment 1.

4.2 Material and methods

Both experiments were carried out plants in the greenhouse at company Agriseeds as Experiment 1. The rehydration experiment began in mid-March 2017 and finished end of May 2017. The Experiment 2 was carried out between mid-March and mid-June, 2017.

4.2.1 Rehydration experiment

Plants in the high-stress blocks of Experiment 1 were rehydrated to field capacity level as for the non-stress treatment in Experiment 1. During the period of April 3rd to May 1st 2017, 2 harvests were carried out, April 17th-harvest 1 (covers period April 3rd -April 17th), May 1st-harvest 2 (covers period April 17th-May 1st) and herbage DM production recorded. Then DM samples of two harvests were pooled, ground and their feed quality (dry matter digestibility, crude protein and metabolizable energy) determined via NIR Systems 5000, Maryland, USA as for Experiment 1. Fertilizer Thrive (All Purpose Soluble fertilizer) was applied once as instructed two weeks before harvest 1.

Illustration 4.1 Overview of the 180 pots under rehydration treatment.

4.2.2 Experiment 2

Experiment 2 had four cultivars (Alto, Barberia, Bronsyn and Trojan) x four replicates with each replicate the mean of three pots, totally 4 x 4 x 3 = 48 pots. They were grown under high-stress water treatment, scales and measuring cup were used to keep water content in all the pots 3 times a week (Monday, Wednesday and Friday).

Three to five tillers of each plant were transplanted to the 4L experimental pots (January 2017). In the first month, all the plants were maintained at field capacity to let them establish in the pots. After that, all pots were let dry to high-stress level (30% field capacity) by mid-March as in Experiment 1.

Two harvests were carried out, May 1st-harvest 1 (covers period, April 10th-May 1st) and May 22nd-harvest 2 (covers period, May 1st-May 22nd). Then the Fresh weight and DM production were recorded as for Experiment 1, except that harvests were carried out every three weeks instead of two weeks. Dry matter samples were pooled, ground and their feed quality (dry matter digestibility, crude protein and metabolizable energy) determined via NIR Systems 5000, Maryland, USA as in Experiment 1.

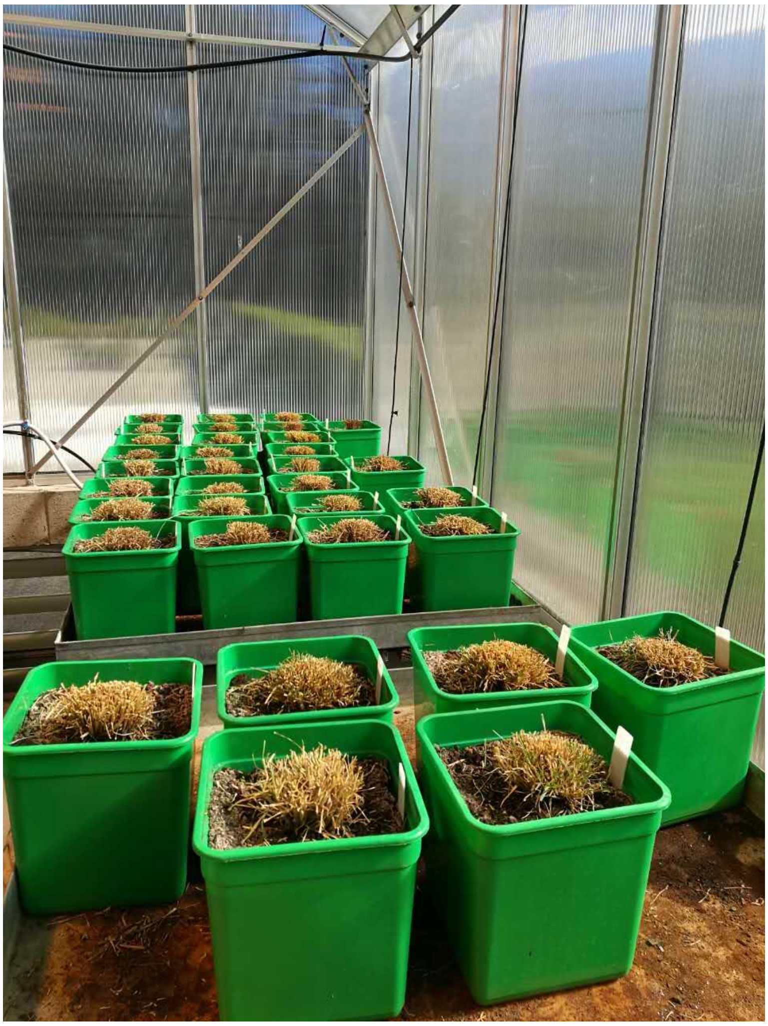

Illustration 4.2 Layout of the small 'repeat' experiment-Experiment 2 in Agriseeds glasshouse.

4.3 Results

4.3.1 Rehydration experiment

In the rehydration experiment, dry matter (DM) production at both harvest 1 and 2, were not significantly different among the five cultivars (P=0.339 and P=0.210, Table 4.1). Dry matter was lower in harvest 2 (grand mean: 2.13 g) than in harvest 1 (grand mean: 3.03 g).

Dry matter digestibility and metabolizable energy were greater for cultivar Norway and Trojan than the other three cultivars (P=0.008 and P=0.025, Table 4.1). However, crude protein was similar among five cultivars (P=0.408).

Table 4.1 Shoot dry matter (mean: DM(g/pot)) production and feed quality dry matter digestibility (DMD), crude protein (CP) and metabolizable energy (ME) of five perennial ryegrass cultivars in two harvests (two weeks apart) in plant Rehydration Experiment on high-stress plants.

		Cultivar					P value
		Alto	Barberia	Bronsyn	Trojan	Norway	
DM (g/pot)	Harvest 1	3.77	2.93	2.98	2.77	2.69	0.339
	Harvest 2	2.56	2.40	2.17	1.78	1.76	0.210
Quality	DMD (%)	70.06	70.01	70.17	71.56	72.47	0.008
	CP (%)	28.30	30.04	27.21	28.49	27.27	0.408
	ME(MJ/Kg)	10.63	10.64	10.60	10.91	10.99	0.025

4.3.2 Experiment 2

There were no significant differences among four cultivars on DM production (P= 0.094 and P= 0.306) at both harvests in Experiment 2 (Table 4.2).

LWC was also similar for the four cultivars at both harvests (P=0.502 and P=0.163) but LWC of Bronsyn was much lower in harvest 2 than in harvest 1 (Table 4.2).

Dry matter digestibility, crude protein and metabolizable energy were similar for all cultivars (Table 4.2).

Table 4.2 Comparison of Fresh weight (FW), Dry matter (DM), Leaf water content (LWC) with four cultivars and feed quality in dry matter digestibility (DMD), crude protein (CP) and metabolizable energy (ME) with three cultivars under the high-stress blocks in Experiment 2.

		Cultivar				P value
		Alto	Barberia	Bronsyn	Trojan	
Harvest 1	FW(g/pot)	2.65	2.43	1.15	4.58	0.108
	DW(g/pot)	0.78	0.64	0.3	1.32	0.094
	LWC (%)	71.58	73.81	76.11	71.64	0.502
Harvest 2	FW(g/pot)	3.43	2.4	1.45	3.72	0.280
	DW(g/pot)	0.88	0.62	0.45	1.12	0.306
	LWC (%)	76.22	73.68	61.96	72.97	0.163
Quality	DMD (%)	75.8	73.21		76.47	0.090
	CP (%)	24.78	24.38		23.65	0.646
	ME(MJ/Kg)	11.66	11.16		11.77	0.089

4.4 Discussion

After five months of the high stress treatment in Experiment 1, all cultivars were rehydrated to field capacity level in the Rehydration Experiment. Dry matter production recovered back to 3.03 g/pot by average at first harvest and this is similar to values at field capacity (non-stress treatment) in Experiment 1. However, the average DM declined to 2.13 g/pot in harvest 2, possibly because nutrients were limiting growth as fertilizer was only applied before harvest 1. There were no significant differences in DM production among cultivars in both two harvests, which was consistent with results of DM production in Experiment 1. A similar ability to recover from drought was reported for 8 perennial ryegrass cultivars (He *et al*., 2017b). Moreover, they found that non-irrigated plants produced similar yield with irrigated plants in the first month after rehydration, and non-irrigated plants had a higher yield than irrigated plants in the second month. Two possible reasons for this finding were given. Firstly, there may have been greater nutrients in the soil which was under drought condition, and when water became sufficient, plants could increase growth (Renkema *et al*., 2012; Yingjajaval, 2013). Secondly, the increased growth may have come from non-structural carbohydrates accumulated in the plants during the drought stress period. The rehydration process may have activated plants to remobilize these non-structural carbohydrates to accelerate the growth under well-watered conditions (Volaire *et al*., 1998; Yang *et al*., 2013). An early study, Korte and Chu (1983) even discovered that the yield of previously stressed plants after rehydration exceeded those under the previously irrigated treatments. They called this phenomenon 'compensatory growth'.

Feed quality was carried out as in Experiment 1. Dry matter digestibility and metabolizable energy dropped back to 'normal' levels, which were similar to results of plants under non-stress blocks in Chapter 3 (Table 3.3 & 3.4; Table 4.1). Also, Trojan and Norway had higher DMD and ME than the other cultivars (P=0.008 and P=0.025). However, CP did not return to normal level but stayed higher than in Chapter 3.

The small 'repeat' experiment (Experiment 2) described in this chapter was carried out to check the results of Experiment 1 in Chapter 3. Only one drought treatment was involved and thus the number of the pots was lower than experiment 1. At first, the five cultivars had 4 replicates with each replicate mean 3 pots (5 x 4 x 3 = 60 pots) were prepared to do this experiment. However, after one-month establishment and two weeks of dehydration, almost all plants of Norway cultivar had dead. Therefore, Norway cultivar was removed from this experiment. When water levels in all pots were uniform and stable, then the drought treatment started. After this, some plants of Bronsyn and Trojan died. Therefore, the number of replicates in Experiment 2 was much lower than originally planned.

Unlike Experiment 1, three weeks instead of two weeks were allowed for growth between two harvests to allow the plant to produce more biomass. However, plants under drought treatment showed very slow growth in the first week, then they had a very slow growth rate in the next two weeks. Therefore, harvest material was not enough for the NIR test. In the case of Bronsyn, material was so small, it was not included in the NIR test. Thus the NIR results are for only three cultivars and do not include Bronsyn and Norway. The DMD, CP and ME were similar to high-stress plants in Experiment 1, but DMD and ME had no significant differences among the three cultivars, this may be because Norway was not involved and sample number was lower than Experiment 1.

This Experiment 2, as a small 'repeat' experiment was carried out to check the findings of Experiment 1. Overall, the results of Experiment 2 were similar to experiment 1 with no significant differences among cultivars on DM production under water stress. However, unlike experiment 1, DMD, CP and ME, the three quality variables were not significantly different across cultivars.

Chapter 5

Final Discussion and Conclusions

5.1 Final discussion and conclusions

Agriculture is a very important sector of the New Zealand economy as it contributes approximately half of the total NZ exports and thus makes a large contribution to the NZ gross domestic production (GDP) (Moot *et a*l., 2009; Statistics NZ, 2017). Farming systems, in particular grass dominant pastures, take up the major proportion of land in NZ. In certain areas like Canterbury, water availability severely limits growth. In Canterbury, irrigation is commonly used in the summer months. Also, changing climate issues such as global warming and rainfall reduction are threatening the agricultural industry. Therefore, drought has become an increasing focus for both plant breeders (Matthew *et al*., 2012) and meteorologists. Moreover, the NZ government has provided funding support for communities to set up irrigation to help farmers deal with the drought problems (Ministry of Primary Industry, 2017).

Perennial ryegrass (*Lolium perenne* L.) is the most commonly utilized grass species in New Zealand pasture due to its high production and rapid establishment, easy management, the capability of being grazed or made into hay and silage, high nutrition and digestibility (75-80%) (Mackinnon *et al.,* 1988; Langer, 1990; Andrews *et al*., 2007; Tonmukayakul, 2009). However, high yield of perennial ryegrass is not persistent during the summer drought period in NZ (Turner *et al*., 2006). Therefore, introduction of new germplasm and improvement of local perennial ryegrass has been critical work for breeders (Stewart, 2006). Arrangements have also led to the introgression of ecotypes from other parts of the world such as France, Morroco and Norway.

In the current study, five cultivars were used. Three of these cultivars (Alto, Bronsyn and Trojan) are local and one cv 'Norway' comes from Norway and Barberia from Morocco. These cultivars were chosen to represent distantly related germplasm from different origins in order to assess the diverse *Lolium* gene pool. Three local cultivars, Alto, Bronsyn, Trojan from separate clusters of the perennial side of the neighbour-joining dendrogram (Figure 2.1) and an extremely distant genotype, Barberia, were chosen along with the Norway ecotype, not represented on the dendrogram but reported to have good water use efficiency (WUE) attributes (Pembleton *et al*., 2016a; Cyriac *et al.,* 2017).

In Chapter 3, the five cultivars were compared under three water treatments (100 % field capacity (FC) as non-stress, 50% FC as mid-stress, 30% FC as high-stress) and their dry matter production, leaf water content, leaf width, photosynthetic rate, stomatal conductance and feed quality (dry matter digestibility, crude protein and metabolizable energy) determined. This was Experiment 1. Chapter 4 had two small experiments. Firstly, plants under the high-stress treatment in Experiment 1 were rehydrated and their subsequent DM production and feed quality determined. Secondly, a small 'repeat' experiment (the four cultivars under the high-stress treatment) was used to check the findings of Experiment 1.

All five cultivars showed similar decreases in shoot DM (g/pot), leaf water content, leaf width, photosynthetic rate per unit area and leaf stomatal conductance (across experiments where applicable) in response to the increased water stress: there was no significant differences between cultivars. This emphasises the crucial functions of water to all cultivars of perennial ryegrass and these results are similar to those related in several previous studies (Cui *et al*., 2015; He *et al*., 2017b; Matthew *et al*., 2017). Matthew *et al.,* (2012) concluded that it was unrealistic to hope to produce a cultivar of perennial ryegrass which maintains high production during the summer drought period in NZ. However, Cyriac *et al*., (2017) observed the variation in drought responsiveness between cultivars. The results obtained here agree with the conclusion of Matthew *et al*., (2012) and indicate that it will be very difficult to develop a perennial ryegrass cultivar with maintained growth under water stress conditions.

In Experiment 1, water stress affected feed quality and this was dependent on cultivar. Across the two harvests, dry matter digestibility and metabolizable energy were greater under the high-stress conditions and also greater for Norway and Trojan than other three cultivars. Further chemical analysis would be required to determine the reasons for these differences. The finding of increased DMD and ME under water stress was consistent with the study of Abraha *et al.,* (2015), which compared annual ryegrass feed quality under different water levels, and found increasing digestibility, ME and CP values under low water levels. However, feed quality was similar for the different cultivars in Experiment 2. Also, the decrease in DM production under water stress is so great that any slight increase in feed quality would make little difference to animal production under water stress conditions.

After five months of the high stress treatment in Experiment 1, all cultivars were rehydrated to field capacity level in the Rehydration Experiment. Dry matter production and feed quality recovered back to values obtained at field capacity (non-stress treatment) in Experiment 1. However, the average DM declined in harvest 2 after rehydration, possibly because nutrients were limiting growth. There were no significant differences in DM production among cultivars in both harvests, which was consistent with results of DM production in Experiment 1. The strong ability to recover from drought was reported for eight perennial ryegrass cultivars (He *et al*., 2017b). Moreover, they found that non-irrigated plants produced similar yield with irrigated plants in the first month after rehydration, and non-irrigated plants had a higher yield than irrigated plants in the second month. Two possible reasons for this finding were given. Firstly, there may have been greater nutrients in the soil which was under drought condition, and when water became sufficient, plants could increase growth (Renkema *et al*., 2012; Yingjajaval, 2013). Secondly, the increased growth may have come from non-structural carbohydrates accumulated in the plants during the drought stress period. The rehydration process may have activated plants to remobilize these non-structural carbohydrates to accelerate the growth under well-watered conditions (Volaire *et al*., 1998; Yang *et al*., 2013). An early study, Korte and Chu (1983) even discovered that the yield of previously stressed plants after rehydration exceeded those under the previously irrigated treatments. They called this phenomenon 'compensatory growth'.

In New Zealand, perennial ryegrass drought tolerance research has focused mainly on screening for drought tolerant differences in dry matter production (Cyraic *et al*., 2017; Matthew *et al.,* 2017). Understanding what the mechanisms of drought tolerance are in perennial ryegrass types is critically important for the outcome required. Turner (1986) defined these mechanisms and whether they interfered with productive processes or not. This needs to be understood by breeders who have to decide to breed for either continued production under drought stress or summer dormancy and recovery. Hussain (2013) and Matthew *et al*., (2017) have made good progress in this area, but translating this knowledge to breeding targets in perennial ryegrass is still in its infancy. The role of endophyte on perennial ryegrass drought tolerance has also been investigated (Cheplick *et al*., 2000; He *at al*., 2017ab) and is another confounding factor that requires consideration. Recent advances in perennial ryegrass genomics have enabled phylogenetic relationships between perennial ryegrass cultivars to be established (Pembleton *et al*., 2016a). This in turn means that a more systematic approach to investigating drought stress differences between perennial ryegrass groups is possible.

The work described in the current study looked at the drought stress response across a range of cultivars that represent distant branches of the perennial ryegrass phylogenetic tree currently used in NZ perennial ryegrass research and breeding. The purpose of this was to see if there were any obvious drought stress response differences that might be exploited by breeding companies and further investigated to understand the mechanism of the response. The results indicate that it will be very difficult to develop a cultivar with maintained growth under water stress conditions. However, against this, perennial ryegrass cultivars, in general, have a strong ability to recover from drought which is an extremely important characteristic in perennial pastures subject to periodic water stress.

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Appendix

Table 1. Leaf dry matter (mean: DM g/pot) production of five perennial ryegrass cultivars over four harvests (two weeks apart), with three water regimes (non-stress, mid-stress and high-stress) with average and standard deviation (SD).

Harvest	water stress	Cultivar					Average	SD
		Alto	Barberia	Bronsyn	Trojan	Norway		
Harvest 1	non	2.82	3.31	2.57	2.54	2.28	2.70	0.39
	mid	2.54	3.18	2.79	2.60	2.53	2.73	0.28
	high	3.08	3.16	3.22	3.00	3.13	3.12	0.08
Harvest 2	non	3.72	4.32	3.71	3.49	3.30	3.71	0.38
	mid	3.25	3.18	3.21	3.19	2.94	3.15	0.12
	high	2.69	2.50	2.69	2.48	2.61	2.59	0.10
Harvest 3	non	3.50	4.60	3.50	3.47	3.33	3.68	0.52
	mid	2.03	1.92	2.00	2.02	1.99	1.99	0.04
	high	0.59	0.60	0.64	0.55	0.71	0.62	0.06
Harvest 4	non	2.83	3.13	2.49	2.88	2.68	2.80	0.24
	mid	2.22	2.28	2.15	2.18	2.13	2.19	0.06
	high	0.99	0.99	0.91	0.90	0.98	0.95	0.05

Table 2. Leaf water content (mean: LWC %) of five perennial ryegrass cultivars over four harvests (two weeks apart), with three water regimes (non-stress, mid-stress and high-stress) with average and standard deviation (SD).

Harvest	water stress	Cultivar					Average	SD
		Alto	Barberia	Bronsyn	Trojan	Norway		
Harvest1	non	83%	84%	84%	81%	83%	83%	0.01
	mid	83%	84%	83%	82%	83%	83%	0.01
	high	83%	83%	82%	81%	82%	82%	0.01
Harvest2	non	84%	85%	85%	83%	84%	84%	0.01
	mid	83%	84%	84%	82%	83%	83%	0.01
	high	81%	80%	80%	80%	80%	80%	0.00
Harvest3	non	83%	83%	83%	81%	82%	82%	0.01
	mid	81%	81%	80%	79%	80%	80%	0.01
	high	78%	80%	79%	78%	76%	78%	0.01
Harvest4	non	81%	82%	80%	80%	79%	80%	0.01
	mid	80%	80%	80%	78%	78%	79%	0.01
	high	75%	77%	77%	75%	73%	76%	0.02