Evaluation of subterranean clover cultivars for New Zealand dryland pastures

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Abstract: Subterranean clover cultivars available in New Zealand have been currently and historically reliant on the Australian market and research. Currently there is very little information available which is related to cultivars performance under a cooler New Zealand climate. This study evaluated 11 subterranean clover cultivars and their performance in mixed swards. On average cultivars throughout this experiment performed poorly in their germination ability, under lab conditions (15°C). Large proportions of these seeds remained hard with the earliest flowering culivar 'Monti' consisting of 67% hard seed, while a later flowering cultivar 'Napier' consisted of 33% hard. Along with hardseededness a small proportion of seed throughout all cultivars were unable to germinate and defined as 'dead', suggesting issues during the development stage of the seeds. 'Narrikup' a mid-flowering cultivar, outperformed all other cultivars during the emergence stage, with 293 seedlings/m^2 on the 5th a March and consisted of a high potential to bury its burrs (7/9) (Nichols *et al.* 2013). 'Narrikup' showed the ability to regenerate and survive throughout the 2015 season, still consisting of a 15% sub clover cover by the end of September. The cultivar 'Antas' showed huge potential in its first year with the highest overall yield in September 2014 (760 kgDM/ha) and highest sub clover content in November 2014 (2710 kgDM/ha). However in the following year 'Antas' performance dropped drastically, with its inability to regenerate. This was attributed to its subspecies family *brachycalycinum*, unlike *subterraneum* and *yanninicum, brachycalycinum* cannot actively burr its burrs and is therefore not protected from the aerial environment, effecting its seed potential to develop seed (Francis *et al.* 1972; Collins *et al.* 1976). Both 'Narrikup' and 'Antas' presents how essential the burial process can be under the cooler New Zealand climate. Older cultivars including both 'Mt Barker' and 'Woogenellup' (late flowering cultivars) began poorly in their ability to regenerate reaching only 47 seedlings/m^2 and 78 seedlings/m^2 respectively by the 5th of March. By the final visual score in spring however these cultivar had recovered with 'Woogenellup'

consisting of around 17% sub clover cover and 'Mt Barker' roughly 10%. 'Denmark' a later flowering cultivar (Nichols *et al.* 2013) also showed an increase in performance by the end of spring with 11% sub clover cover. Currently studies throughout New Zealand have claimed late flowering cultivars will out compete both mid to late flowering cultivars (Chapman 1992; Collins *et al.* 1976; Smetham and Ying 1991; Sheath and Richardson 1983), under this experiment however this was not an obvious trend.

Keywords: Germination, hardseededness, regeneration, establishment, dryland, persistance, subspecies, flowering, burr burial

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1. Introduction

Throughout pastoral systems in the South Island of New Zealand, the contribution of legumes to the fixation of N is essential for many farming systems (Lucas *et al.* 2010). Legumes in the pastures ensure nitrogen transfer to the grasses which is crucial to ensure high DM production and increasing grazing preference for pasture through increased nitrogen contents. In return legume content throughout the pastures can play a key role in promoting live weight gains especially in lambs (Ates *et al.* 2012).

Although white clover *Trifolium repens* is the traditional legume used throughout New Zealand, many areas in the country can have significant limitations in the growth of legumes due to summer dry conditions. Other contributing factors including soil fertility, grazing and temperature can make it extremely difficult in summer dry areas to maintain the traditional white clover based pasture. Therefore requires different legumes to provide appropriate feed for grazing animals (Chapman *et al.* 1986). Subterranean (sub) clover (*Trifolium subterraneum*) is a winter active (Widdup & Pennell 2000), autumn germinating legume which evolved in Mediterranean climates of hot-dry summers and cool-warm, moist winters (Smetham & Ying 1991). This clover species has been found to have one of the greatest potentials throughout New Zealand's seasonally dry areas (Chapman *et al*. 1986).

Sub clover was widely used throughout New Zealand within drought prone areas in the 1940's. However due to the lack of cultivar regeneration in pastures and uneven distributions throughout different regions due to poor management, frequent droughts and lack of re-sowing and understanding of cultivars, sub clover became less popular (Widdup & Pennell 2000). Currently there is a wide range of differences between sub clover cultivars which can be used throughout New Zealand. Sub clover cultivars differ in their hardseededness, flowering, burr burial, winter productivity, soil tolerance and growth forms.

Historically and currently, the sub clover cultivars which are available to New Zealand have been hugely reliant on the Australian cultivars, seed harvest and availability to export. Plant Variety Rights (PVR), trans-Tasman seed company loyalties, New Zealand biosecurity issues with weed seed, soil contamination and the perception that the New Zealand market for sub clover seed was not large enough to bother with, have been important influences on sub clover imports. This has resulted in the selection of cultivars on the New Zealand markets being based around Australian data, rather than New Zealand field experiments (Lucas *et al.* 2015).

'Mt Barker' has had an early importance throughout New Zealand and overtime under the New Zealand climate and grazing management has become well adapted along with 'Tallarook' (MacFarlane & Sheath 1984). In initial studies in the 1980s which were conducted over a wide range of sites showed that 'Mt Barker' and 'Tallarook' were the most predominate cultivars over the 8 New Zealand sites (Chapman *et al.* 1986). 'Tallarook' however is a very late flowering cultivar which lead to poor seed sets under the driest conditions (600mm annual rainfall). In a test conducted on 21 farms throughout North Island hill country it was also concluded that 'Mt Barker' and 'Tallarook' were the best adapted cultivars. Recommending that until Tallarook-type characteristics became available on the market, initially 'Mt Barker' and 'Tallarook' were the only two cultivars they would recommend on North Island hill country (MacFarlane *et al.* 1990).

In the more modern experiments which have taken place in New Zealand it has been found that the new Australian cultivars have improved seed set, and shown a better potential through regeneration and herbage yield compared to the older cultivars. Throughout the Canterbury region the late-maturating cultivars have been found to be best adapted, with 'Denmark' and 'Leura' re-establishing more seedlings through grazing trials (Widdup & Pennell 2000). Costello and Costello (2013) found that the original cultivar used throughout New Zealand 'Mt Barker' is still found through many hill country pastures. The late flowering 'Leura' however was found to have high potential on the flats in Canterbury. Under the 'maxclover' trial at Lincoln University sub clover 'Denmark' and cocksfoot mixes were found to produce a more reliable yield in dry years than white clover and ryegrass (A. Mills *et al.* 2008). This shows there is a range of possibilities when deciding which sub clover cultivar can be used throughout New Zealand.

This dissertation investigates and explains some preliminary findings under a two year dry land, Lincoln University field experiment, on how a range of different sub clover cultivars perform under the Canterbury climate. It aims to identify the possibility of using 'new' cultivars which have not previously been examined/ or examined in depth under the New Zealand environment. With the specific objective of looking at 11 sub clover cultivars sown with cocksfoot in March 2014, and their ability regenerate and perform under the New Zealand climate. Along with identifying what cultivar traits are contributed to the best performing cultivars.

2. Review of Literature

2.1 Introduction

Legumes play a key role throughout a range of pastoral systems within both the South Island and North Island of New Zealand. In many areas where the use of nitrogen (N) fertiliser i.e. urea may not be deemed cost efficient, farmers can rely heavily on legumes such as clover to incorporate N into the pastoral system (Lucas *et al*. 2010). The addition of N from the clover can insure nitrogen transfer to grasses, which is crucial in ensuring the development and growth of a sward. With increased nitrogen content in grasses grazing preference can increase, higher quality and higher dry matter is produced, in return playing a key role within live weight gains of livestock (Ates *et al*. 2012).

By tradition New Zealand's pastoral systems preference, has been the white clover within a sward. However due to limitations including fertility, rainfall, summer droughts, temperatures and acidity of soil white clover can be difficult to maintain in some dryland systems. Sub clover has been found to have one of the greatest potential in areas which have these difficulties (Chapman *et al*. 1986). Sub clover is a winter-active (Widdup & Pennell 2000), autumn germinating legume which is adapted to a Mediterranean climate of hot-dry summers and cool-warm, moist winters (Smetham & Ying 1991). The clover was widely used throughout drought prone areas in the 1940's, consisting mainly of the cultivars 'Mt Barker' and 'Tallarook'. However with the lack of sub clover regeneration in pastures and uneven distributions throughout different regions due to poor management and understanding of cultivars, continuous droughts and lack of re-sowing, sub clover became unpopular (Widdup & Pennell 2000).

When using sub clovers in dry areas many factors including seed dormancy, hardseededness and flowering, time should be taken into consideration when choosing a cultivar to suit a New Zealand climate. Currently the main sub clover cultivars which persist throughout New Zealand dryland systems consist of later flowering cultivars 'Mt Barker' and 'Tallarook'. The regeneration of these populations can be significantly jeopardised through intensive grazing during re-seeding and unfavourable weather conditions which can lead to "false strikes" resulting from frequent summer rain followed by dry autumns (Sheath & MacFarlane 1990). Therefore it is essential to understand the properties of sub clover cultivars in order to make an informative decision on which cultivar will persist, which is the basis of this literature review.

2.2 Sub clover characteristics

Table 2.1 Agronomic data for Australian subterranean clover cultivars which have been sown in New Zealand. Data from long-term means of irrigated plants from an early May sowing in Perth, Western Australia (adapted from Nichols *et al*. 2013). Seeds sown/m^2 is a bare seed equivalent rate.

Year = Year seed first sold/ date registered as an Australian cultivar Subspecies: B, brachycalycinum; S, subterraneum; Y, yanninicum. Min. growing-season length (months) is the minimum target environment for reliable seed set. Burr burial: 1, little or no burial; 9, strong burial. Relative hardseededness: 1, least hard; 10, most hard, based on laboratory screening in a diurnally fluctuating 60/15°C temperature cabinet for 16 weeks, using the procedure of Quinlivan & Millington (1962

Cultivar	**Year**	**Subspecies**	**Day to first flowering**	**Min. growing season length (months)**	**Burr burial rating (1-9)**	**Hardseededness (1-10)**	**Seed Weight (mg)**
Mt Barker	1900	S	137	7.5	3	1	8.3
Tallarook	1936	S	163	9	5	1	7.4
Woogenellup	1959	S	130	7	3	1	10.7
Seaton Park	1967	S	110	5	7	5	9.1
Trikkala	1975	Y	112	5.5	6	2	12.3
Karridale	1985	S	139	7.5	6	2	7.9
Denmark	1992	S	142	7.5	5	2	7.1
Gosse	1992	Y	126	7	5	3	11.0
Goulburn	1992	S	141	7	5	5	5.1
Leura	1992	S	147	8	5	2	7.4
Antas	1999	B	138	7.5	1	3	10.0
Campeda	1999	S	123	6	6	5	8.1
Napier	2001	Y	140	7.5	6	5	11.4
Coolamon	2003	S	133	6.5	7	5	7.7
Narrikup	2009	S	126	6.5	7	3	5.4
Rosabrook	2009	S	142	7.5	6	5	6.2
Monti	2013	Y	110	5.5	6	2	9.9

Sub clover is an annual legume and divided into three main sub species; *brachycalycinum, subterraneum* and *yanninicum.* Naturally this specie of clover is distributed around Mediterranean regions, predominantly with wet winters and dry summers. Water requirements for ecotypes of this clover to complete their life cycle can range from 350 – 1100mm depending on the cultivar and therefore it can be expected to exhibit wide variations in many of its attributes. (Morley 1961). Cultivars can and will therefore vary significantly through a wide range of attributes including subspecies, flowering, the ability to bury burrs, hardseededness and seed weight (Nichols *et al.* 2013). All of these traits can in return effect how particular cultivar will persist under different climates (Morley 1961) and it is essential to understand what each of these factors mean and how they can effect sub clovers performance.

2.3 Subspecies

There are 3 subspecies of sub clover; *subterraneum*, *yanninicum* and *brachycalycinum*. Under their natural habitat (Mediterranean climates) subspecies *subterraneum* and *yanninicum* are commonly found in neutral to slightly acidic soils, whereas the sub specie *brachycalycinum* will be found in neutral to slightly alkaline soils (Bolland 1987). All subspecies have been found to have the capability to persist in light soils (i.e. silt loams) where it can prove difficult to maintain other legumes including the traditional white clover. Under farming conditions *subterraneum* has been found to persist and thrive under soil pH of 5.5 – 7.5, whereas *yanninicum* has adapted more to water logged soils and can survive under poor drainage (Costello & Costello 2013).

Under a study performed at Kaikohe, New Zealand, 9 sub clover cultivars were compared under grazing in a hill pasture. The cultivar 'Clare' represented the sub specie *brachycalycinum* and was high producing in the first year. Yet 'Clare' was unable to regenerate in the second year due to its inability to bury its burrs. The sub specie *subterraneum* cultivars 'Mt Barker' yielded less than the *yanninicum* species "Trikkala' and 'Larisa', 63% and 165% respectively over the four years (Table 2.2) (Sheath *et al.* 1990). The higher productivity of 'Larisa' and 'Trikkala' can be attributed to the sub specie *yanninicum ,* as it is suited to winter-wet soils (Costello & Costello 2013), which occurred regularly throughout this study (Sheath *et al.* 1990).

Table 2.2 Subterranean clover yields (kg DM/ha) from 13cuts over 4 years (% of whole sward yield in parentheses), and clover and total sward yields relative to 'Mt Barker' (Sheath *et al.* 1990).

		Relative yields (%)	
Cultivar	**Subspecies**	**Clover**	**Total**
Clare	B	85	94
Mt Barker	S	100	100
Trikkala	Y	163	106
Larisa	Y	265	119

With the current information available it is clear to see which sub species will perform under particular soil conditions (i.e. *subterraneum* under acidic soils) and/or climates (i.e. *yanninicum* under winter wet conditions). With the current research it can allow for a farmer to select a sub specie which will be best adapted to their farms environment. From this it will also allow farmers to then select a cultivar within a sub specie dependent on a range of other traits which can benefit performance.

2.4 Flowering of sub clovers

Autumn, winter and early spring herbage production from sub clover is highly dependent on the seedling density. In Mediterranean environments which can experience a sudden end to rainfall, one of the most essential determinants of seed production and the success of sub clover, is the time at which flowering begins relative to the onset of dry conditions in the summer months (Smetham *et al.* 1994). Rossiter (1959) also concluding that a strong relationship occurs between seed yields and the number of days to the first flowering had a significant effect on sub clovers production. However in unpublished work from M.L. Smethan between 1968 to 1969, failed to identify a relationship between 37 sub clover cultivars, flowering dates and improved yields, when grown in shallow soils, in cool Canterbury temperatures (Smetham *et al.* 1994).

Flowering is an essential characteristic of sub clover, as it determines how much seed will mature prior to the dry season. Early flowering cultivars, have been known to mature a large quantity of seed that exhibit high levels of hardseededness following maturity (although this can also depend on the genotype). Early flowering cultivars are therefore regularly found to be unsuitable for the New Zealand climate, which is attributed to the requirement of high fluctuating temperatures to break down hardseededness and allow germination to occur (Smetham & Ying 1991).

Within a nationwide evaluation on the persistence of sub clover cultivars on varying hill country, on average late-flowering cultivars were superior over both early or mid-flowering cultivars (Chapman 1992). Only in extremely dry environments including North Canterbury were moisture deficiency would commonly occur before November, were late flowering cultivars at a disadvantage (Hoglund 1990). Although flowering date has a significant influence on cultivar performance, it should be noted that it is the traits associated with the flowering dates which leads are what lead to these improvements i.e. the difference in flower and/or stem growth and burr production. Later flowering plants have a greater opportunity to exploit favourable spring growing conditions and therefore leading to improved performance and persistence under a New Zealand environment (Chapman 1992).

Smetham *et al.* (1994) found that under cool climatic conditions (Christchurch, New Zealand), the highest yields resulted from the late-flowering cultivars. However in 1993 drought onset appeared to be a month earlier than typical, disadvantaging the early flowering cultivars significantly. The number of days of flowering prior to the drought in mid-October was found to negatively correlate with seed yield (Table 2.3) due to the early flowering cultivars failure to mature seed. Later flowering cultivars (which flowered just before the drought) conversely were able to survive or recommence flowering. Characteristics which had the highest relationship to seed yields included;

flowering after the drought, total days of flowering and days of recommenced flowering after drought, showing how essential flowering can be within sub clover cultivars performance.

Table 2.3 Estimates of correlation between aspects of the flowering pattern of subterranean clover and subsequent seed production in 1993. (* represent significance)

Aspects of flowering	**Correlation coefficent**
Days to first flower appearance, from 30 August	0.443 *
Days of flowering before drought	-0.425 *
Days of flowering after drought	0.677 *
Days of maximum flowering before drought	-0.330 *
Days of maximum flowering after drougtht	0.402*
Days of recommenced flowering after drought	0.649 **
Days (total) of flowering	0.576*

Each individual cultivars within sub clover will have variations in its growing seasons and time to first flowering. Table 2.1 shows how much variation can occur within the range of sub clovers which have been/are available within New Zealand. Mt Barker which is the oldest cultivar, has a late flowering date, with 137 days till first flowering and 7.5 months to complete its growing cycle. Whereas Monti, which may be very similar in sight (hairy runners, red strip on stipule and brown flecks on leaves), is a new cultivar with a short growing cycle of only 5.5 months (Nichols *et al*. 2013). With information available it currently provides a relatively reliable assumption that under majority of the New Zealand climate conditions the later flowering cultivars will outperform both the early and mid-flowering cultivars.

2.5 Burr Burial

Burr burial in sub clover is a specialisation known as geocarpic (ability to produce or ripen below the ground surface) which is well developed in most subspecies and cultivars. The mechanism of burial occurs after pollination of the floret, were the peduncle will turn downwards and elongate, pressing its tip into the soil (Francis *et al.* 1972). Burr burials main function in sub clover cultivars is to protect the developing seed from unfavourable conditions. It has been found to be a means of protection from the formation of the seed at its earliest stages of development until close to maturity. Which in return results in cultivars which can vary their size/weight of burrs and seeds, significantly more seed formed per burr and higher seed viability (Collins *et al.* 1976).

The variation of burr burial between cultivars can be largely dependent on which sub specie a cultivar belongs to. The subspecies *subterraneum* and *yanninicum* have the ability to actively bury their burrs. Their peduncle become elongated and thicken allowing for them to actively push their burrs into the ground, becoming anchored by the sterile calyces which form the burr structure. The sub specie *brachycalycinum* however is known for having long thin sarmentous peduncles which spread until they find a suitable dark habitat (Bolland & Collins 1986), such as under stones or cracks which have formed in the soil but they cannot actively bury its burrs (Bolland 1987).

With the information available in relation to burr burial, it appears that when you are selecting for a cultivar a higher burr burial will lead to higher seed numbers which are larger and more viable. Allowing for farmers throughout New Zealand to select based on the subspecies and cultivars for the potential to bury burrs.

2.6 Germination and hardseedness

Germination within sub clover species is controlled by two main mechanisms; the embryo dormancy and hard-seededness (Walker 2011). Subterranean clover consists of a hard seed coat to prevent the imbibition of water that begins the process of seed germination. The hard seed coating is thought to prevent seeds from becoming imbibed, failing to germinate under unfavourable conditions and stop moulding from occurring (Knight *et al.* 1982).

Germination is often used loosely in scientific literature and it is therefore essential to clarify this definition. Germination begins with in imbibition of a seed and ends with the emergence of an embryonic axis, most commonly the radicle which penetrates its surrounding seed coat structure. Quiescent is known as a mature yet dry seed with a low moisture content between 5-15% and minimal metabolic activity (sub-clovers embryo dormancy stage). In order for germination to occur, water must penetrate the seed while it is in it quiescent stage under suitable conditions (Bewley *et al.* 2013).

For subterranean clover germination relies on the fluctuation of temperatures between 15-35^{0}C (Figure 2.1) to breakdown the hard seed coat and allow the penetration of water (Smetham & Ying 1991) . Upon a viable seeds imbibition of water a chain of events will begin to occur, resulting in the emergence of the embryo signifying a successfully germination. The events occurring following imbibition are complex; upon water entering the seed, metabolism must be initiated which permits recovery of any structural damage caused by the maturation, drying and oxidation of the seed which may occur while dry. Basic cellular activities must be re-established, with the development of the embryo for the preparation of emergence and early seedling growth. This can result in it being difficult to distinguish events related to germination and those linked to other seed changes that take place (Bewley *et al.* 2013).

Figure 2.1 shows the relationship between cultivars germination and their hardseededness. When a sub clover cultivar has a high level of hard seed, the germination process in some cases can become less effective and result in several issues particularly throughout the New Zealand environment (Sheath & Richardson 1983). When looking at this graph it shows that a cultivar with a high level of hard seed, such as 'Howard' (40% hard seed), germination is up to 45% lower than a cultivar with a softer seed such as Woogenellup (5% hard seed).

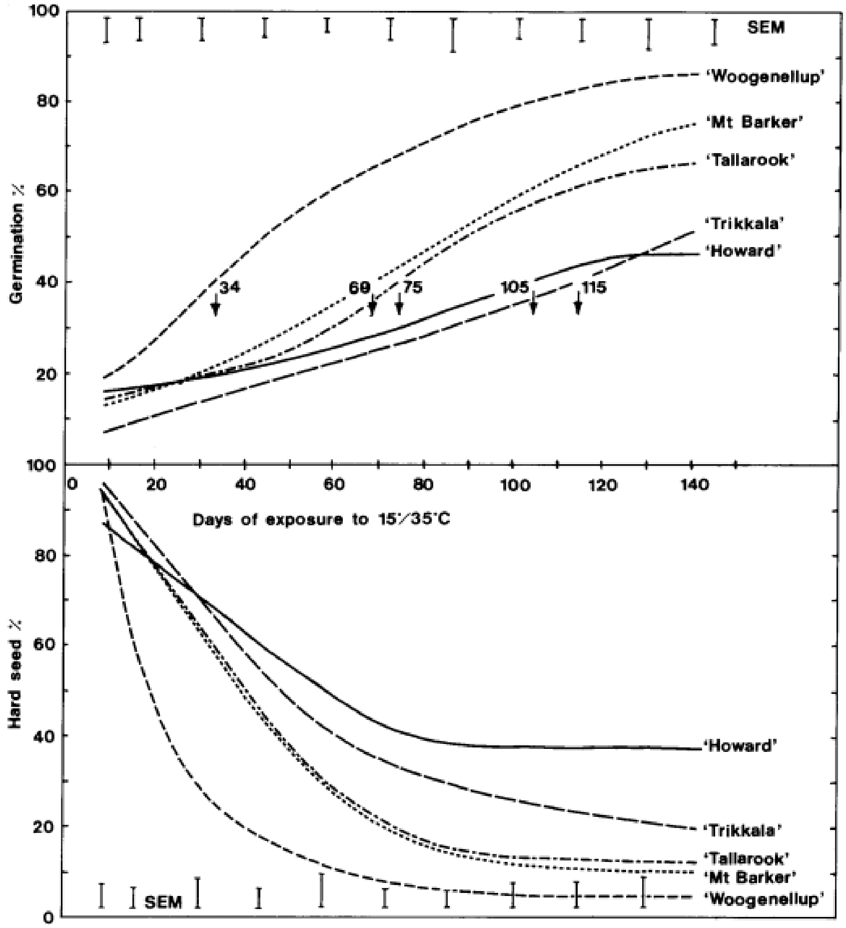

Figure 2.1 Change in germination and hardseededness of seeds of five cultivars of subterranean clover with increasing period of time exposed to 15°C alternating with 35°C every 12 h (Smetham & Ying 1991).

Subterranean clover, like many legumes, has the ability to produce a hard seed which stops the absorption of water and thus germination for periods ranging from weeks to years. The hardseededness development allows the clover species to survive unfavourable seasons caused by both drought and pest attacks, which would otherwise result in failed establishment. Furthermore this mechanism has been known to prevent premature germination which can occur frequently in an environment that differs from a true Mediterranean climates following summer rains (Smetham 2003a).

Taylor (1984) found that after a drought seed losses caused by “false strikes” over a 12 year period, resulted in average losses of 24% of all seed set. Within the New Zealand climate, Dodd *et al.* (1995) measured loss of seed caused by false strikes to be between 4-55% each year between cultivars. This shows the variation that can occur in different climates, which can lead to the failure of subterranean clover establishment in New Zealand.

The level and condition of the hardseedness of subterranean clover is dependent on the cultivar and their genotype (Table 2.1). Hardseededness is obtained after a moisture drop within the seed to below a critically low level. As the seed dries the testa begins to shrink and becomes water resistant at a moisture content of roughly 14% of the dry weight. At this stage the structure at the point of attachment of the seed and the longitudinal groove at the pit of the hilum surrounding (Figure 2.2) act as a hygroscopic valve, allowing moisture out while preventing the penetration of water. The moisture content finally reaches equilibrium when exposed to the driest condition. Subterranean clover seeds need to reach this level of moisture in order to reach an irreversible impermeability (5-7%).

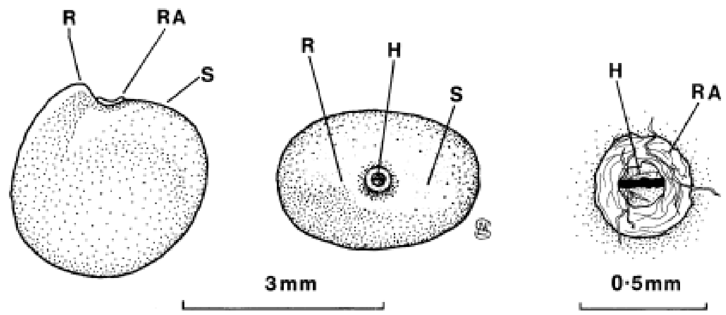

Figure 2.2 Subterranean clover seed in elevation (left) and plan view (middle). The right drawing shows an enlarged hilum (H), with its deep longitudinal groove, surrounded by raised rim-aril structure (RA). The position of the tip of the radicle within the seed (R). The position of the strophiole, under the seed coat (S) (Smetham 2003a).

Under the New Zealand climate particularly in the cooler South Island conditions there has been issues with the levels of hardseededness between cultivars occurring in the autumn months resulting in poor regeneration of clover (Smetham and Ying 1991; Sheath and Richardson 1983). The issue is believed to arise from the larger fluctuation of temperatures which occur in the Australian environment in comparison to that throughout New Zealand (Smetham and Ying 1991).

Within subterranean clover there is an additional mechanism which can contribute to the penetration of water and germination of the seed. The presence of a continuous layer known as the suberin is laid over the coloured part of the seed coat. In the occurrence of moisture stress during the maturation of the seed the suberin layer may be thinner and in some conditions discontinuous. Therefore in conditions where there is soil moisture deficiency following the beginning of flowering the seed may lack the protection of its hard seededness, which leads to seeds failing to germinate following imbibition (Smetham 2003a).

Table 2.1 shows a range of Australian cultivars and their hardseedness within an Australian environment (Nichols, Foster *et al*. 2013). All of the cultivars selected from this table are below 6 in the seed hardness, where 10 is a 'hard seed' and 1 is a 'soft seed', and therefore are more likely to be reliable in the New Zealand environment (Sheath & Richardson 1983). However as this is an Australian table care should be taken when interrupting this data as it could be significantly different under the New Zealand climate and results are more likely than not to vary.

The breaking down of the hardseedness is dependent on the fluctuation of temperatures (Smetham & Ying 1991). With the exposure to sufficient fluctuations between day and night temperatures, the seed hardness will steadily decline in the seed between maturation and the following autumn. The softening of this seed allows for germination to occur. Softening has been found to occur at its highest rate in temperatures between 60°C days and 15°C nights in comparison to the Western Australia temperatures of 46°C days and 15°C nights, however there was no increase in softening when exposed to temperatures of 73°C during the day (Smetham 2003a).

When exposed to cooler conditions for the summer months the softening process will slow. Currently there is evidence of 12-31% of the original seed remaining hard when exposed to cooler Tasmanian summers, such as New Zealand summers, with monthly maximums of 20-23°C and minimums of 10-11°C. Hagon (1971) found that temperature variation was responsible for the softening of the seed because it caused expansion and contractions of the tissue comprising the "strophiole, which lead to a rupture and chemical degradation of the testa." This allows moisture to enter the seed but only at this point resulting in a swollen fleshy addition to part of the ovule and resulting in a negative impact on germination (Smetham 2003a).

Table 2.4 Hardseedness of nine subterranean clover cultivars after exposure to temperatures in Western Australia and 60°C days and 15°C nights, adapted from (Quinlivan & Millington 1962).

		% Hard	
Cultivar	**In early summer, Western Australia**	**After 4 months exposure to summer temperatures, Western Australia**	**After 6 month exposure to alternating 15°C night/60°C days**
Geraldton	97.0	64.7	29.3
Dwalgannup	96.5	62.4	10.3
Burerang	82.4	52.4	28.8
Camamah	95.5	51.2	8.5
Morocco	80.7	35.3	26.4
Woogenellup	86.0	34.4	2.8
Palestine	89.2	29.6	3.1
Basshus Marsh	71.0	16.5	0.2
Mount Barker	58.8	11.1	2.1

Solid information is provided in the seed protection mechanisms and how they work to prevent or establish germination is widely accessible and covered in depth. However the information that is available in relation to this is largely based on Australian data. With New Zealand having a different climate the question arises as to whether this information is actually reliable when used under this climate. As New Zealand climate is known as temperate then it is likely that there will be more severe restrictions to the germination and emergence in this species. In order for New Zealand farmers to select a cultivar effectively, significantly more New Zealand data is required as recommendations currently are based on Australian data.

2.7 Seed Weight

Sub clover is known for its large seed size production, allowing quick establishment and early growth and development in the field (Smetham 1968). Sub clovers dry weight through its early vegetative growth is hugely dependent on the seed size (Figure 2.3), which can vary widely between cultivars (Black 1957). Seed weight can also be largely dependent on the environmental growing conditions and can be significantly affected by both climate (i.e. early droughts tend to lead to smaller seeds) and through management (Ates *et al.* 2006).

Leaf area development in sub clover is also largely dependent on the seed size, with critical leaf area index being reached first by cultivars from large seeds, followed by plants from smaller seeds. A leaf system which is therefore capable of intercepting light energy early will lead to higher dry matter production. However under sward conditions with strong competition for light dry matter production is reduced due to the increase of mutual shading. This means that once the clover has reached its critical leaf area the relative growth rate can be reduced, resulting in little effect in seed size on the final dry matter production of sub clover cultivars (Black 1957).

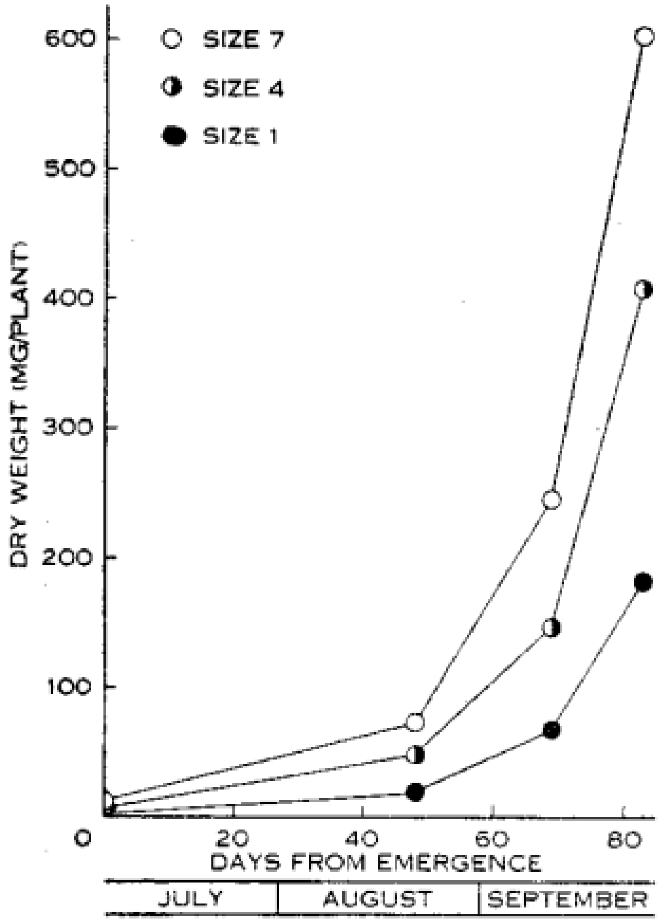

Figure 2.3 The effect of seed size on plants dry weight from day 0 to 80 days from emergence (Seed size 1 = small seeds, Seed size 7 = large seeds). (Black 1957)

From the current information available it appears that selecting on seed size will be beneficial for its sub clovers cultivars during initial establishment. However larger seeds may not lead to significant differences with the final dry matter yields of cultivars under conditions where light is being intercepted by the surrounding the surrounding sward.

2.8 Temperature effect on subterranean clover germination

Within the New Zealand environment the practice to establish subterranean clover and ensure its regeneration is to sow the clover based around the autumn rains. Timing of these rains throughout the New Zealand climate can be exceedingly variable ranging from January through to May. Subterranean clover has the potential to germinate at a quicker rate than other pasture species which are generally sown in cooler temperatures of around 15°C, where the clover will reach a max germination rate of 0.35/d at 15°C. Upon exceeding the maximum temperature germination rate will begin to decrease and even halt as temperatures exceed the maximum (Black *et al.* 2003).

Temperature effects on germination rate and other plant development stages can be quantified in thermal time (Tt) or degree days (ºCd). It is assumed that in conditions where water is non-limiting the appearance of the seedling leaf, stolon, tillers or runners will be regulated by Tt and is the determining factor of a species successfulness (Black *et al.* 2003). Thermal time is based around the plant's development and temperature relating to the degree days which are required between two development stages. The simplest model of thermal time is the mean temperature minus the base temperature.

Different cultivars of sub clover vary in their thermal time requirements for germination (Moot *et al.* 2000). This allows for farmers to select appropriate cultivars for their particular climate. Table 2.5 shows that within a cooler environment such as Mid-Canterbury the use of "Mt Barker" would be sufficiently more effective than the use of "Woogenellup" which requires 33°Cd more units in order for germination to occur and may be more useful in the warmer areas of New Zealand, such as Marlbourgh or the North Island.

Table 2.5 Estimates of base temperatures (T_b) and thermal time (Tt) required for a range of development stages occurring within subterranean clover cultivars (Black *et al.* 2003; Monks *et al.* 2009; Moot *et al.* 2000; Murray 2012).

Cultivar	**Germination**		**First Leaf**		**Stolon Initiation**		**Reference**
	Tb (°C)	**Tt (°Cd)**	**Tb (°C)**	**Tt (°Cd)**	**Tb (°C)**	**Tt (°Cd)**	
Campeda			1.6	194	-0.7	455	Black *et al*, 2003
Denmark			1.1	207	-0.2	426	Black *et al*, 2003
Leura	0.1	61	1	235	-1.3	492	Black *et al*, 2003; Monk *et al*, 2009
Mt. Barker	0	45					Moot *et al*, 2000
Napier			0	174			Muarry, 2012
Rosabrook			0	175			Muarry, 2012
Tallarook	0	48					Moot *et al*, 2000
Woogenellup	0	78	0.1	235	-0.7	442	Black *et al*, 2003; Moot *et al*, 2000

Subterranean clover requires more thermal time for germination than many other legumes with lighter seeds. For example in a study performed by Monk *et al*. (2009), subterranean clover required 50^{o}Cd whereas white clover which has a 1000 seed weight of 0.6g, required 40^{o}Cd and arrowleaf clover only required only 18^{o}Cd. Annual clovers, including subterranean clover, declines as the temperature drops (>30°C), more so then perennial legumes (>35°C). Typically all sub clover cultivars will germinate in the autumn following sufficient accumulation of heat units after either sowing or re-seeding buried seeds, given that the hardseededness has been broken. Vegetative growth will then continue through winter and spring when the reproductive stage begins. This allows for a clover crop to avoid being lost during extreme drought conditions as sub clover will be in its dormant stage in the summer season.

Under Canterbury conditions the soil surface can reach a maximum in February averaging at 38^{o}C and an average daily temperature of around 22°C. With the potential of these soil temperatures to slightly increase in the coming years due to the earth surface warming, this clover specie may become an essential and effective legume within areas of New Zealand which consist of this climate, providing that sowing is occurring at the correct time (Monks, Sadatasilan *et al*. 2009).

Significant amount of information is available on what this clover species requires to grow and develop. However there is lacking in information on individual cultivars that are available within the New Zealand market. This means more data is required, as to which cultivars will survive best under particular New Zealand climates. Although more information is definitely required it does shows there is a real potential for subterranean clover throughout the drier areas in New Zealand.

2.9 Management and regeneration in a farming system

The regeneration of subterranean clover can be affected by climate, grazing styles, closing dates and stocking rates (Sheath & MacFarlane 1990). It is essential for dryland farming throughout New Zealand to identify ways to achieve and maintain a high legume content in spring pastures through both cultivar selection and management. The transfer of nitrogen from legumes to grasses is essential in the dry matter (DM production) and promoting live weight gains (Ates *et al.* 2012). In order to achieve the maximum desired DM production when regenerating sub-clover seedling population densities should be siting between 500-1000 seedlings/m^2, allowing for the sward to receive maximum benefits of the clover (Ates *et al.* 2012).

Currently the recommendations for regeneration and management for sub clover cultivars is based around farmers experience more than research. Within a moist spring condition, set stocking of sub clover dominant pasture is recommended to maintain a pasture mass of 2000 kg DM/ha (Ates *et al*. 2006), to assure adequate seed production (Costello & Costello 2013). However in conditions where spring rainfall is limited grazing the pasture can result to less than 1400 kg DM/ha and rotational grazing of paddocks may be required for some cultivars including ‘Woogenellup’ and ‘Karridale’ (Ates *et al*. 2006).

Spring population of sub clover can be low in dryland pastures due to both the environmental factors and management strategies. As flowering of this clover occurring during the main flush of herbage production and high feed demand from lactating stock, challenges can occur in how to manage this pasture effectively. The conflict arises in relation to the production of seed and the effect the stocking rate can have on the persistence of sub clover (Ates *et al.* 2006). In order to maintain sufficient clover content closing dates of pasture should be taken into consideration (Ates *et al*. 2012). However it should be noted however that cultivars have a range of flowering dates and maturities (Nichols *et al*. 2013) which could lead to variation in how these cultivars are treated.

In a pasture which contains low clover content then the possibility of closing the pasture at an earlier date than the norm may allow for a more efficient regeneration of both 'Leura' and 'Campeda'. By closing the pasture at an earlier date it can prevent the stock from consuming seed burrs, leaves flowers and runners, replenishing the seed bank and increasing seed production. In Figure 2.4 it presents clear evidence that closing a pasture at an earlier date there is a significant increase in regeneration. When closing the pasture on the 10th October in the low stocking rate compared to the 5th December we can see that there is around 900 seeds/m^2 more in the earlier date than the late (Ates *et al*. 2012).

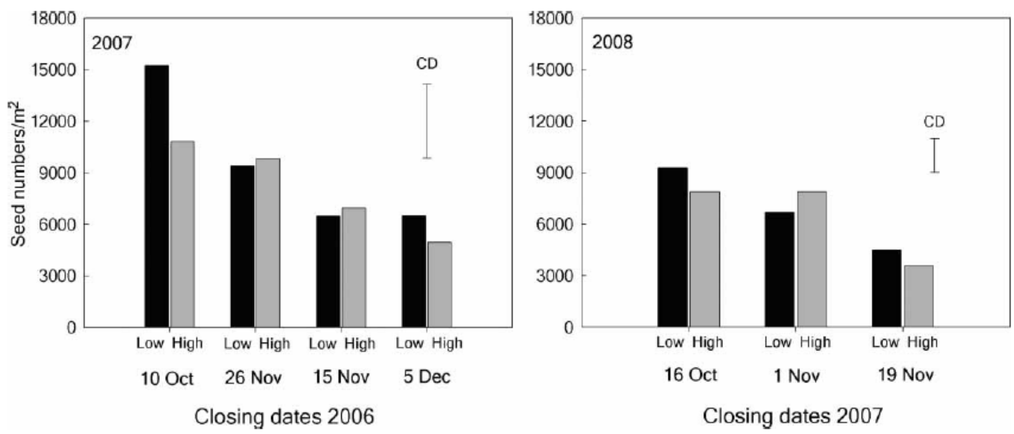

Figure 2.4 Effect of stocking rate and closing dates on regeneration of subterranean clover (Ates *et al*. 2012)

Stocking rate is another strategy which can lead to the increase of clover regeneration. With a lower stocking rate, there is less consumption of burrs, leaves, flowers and runners allowing for greater production of seeds. The 10th October, 2006 the lower stocking rate allowed for 1500 seed/m^2 whereas the higher stocking rate only produced 1000 seeds/m^2 (Ates *et al*. 2012). However this is only taken into consideration two cultivars 'Campeda' and 'Leura' and other cultivars may react differently under these management systems.

Under a rotational grazing treatment during the spring, 'Woogenellup' was found to be unsuited under dryland hill country in Whatawhata, due to low reseeding ability and/or the occurrence of false strikes. Although in the study it still produced good yields, this is likely to have only occurred due to the re-planting (Table 2.6). 'Mt Barker' however was found to be one of the more successful cultivars consistently regenerating under both rotational and continuous grazing, along with having a good dry matter yield but not requiring a high number of replanting (MacFarlane and Sheath 1990).

Costello and Costello (2013) found that within the Hawarden basin, under hot and dry conditions, with lighter soils that 'Leura' a late flowering sub clover had real potential. Although initial grazing of this cultivar had some effect on seedling counts after 5 years of set stocking Leura had begun to show high levels of regeneration. In comparison Denmark was found to have a higher strike rate than Leura from the first year. Both of these cultivars under cattle grazing had a seedling survival of 70% allowing for high regeneration in the following years. Furthermore early to mid-flowering cultivars of an erect habit including 'Clare' and 'Woogenellup' have been found to show extremely poor persistence under hill grazing systems, due to the damage caused during their reproductive phases. The later flowering cultivars of a prostrate habit including 'Mt Barker' and 'Tallarook' have shown much greater ability in their reseeding and regeneration (Dodd *et al.* 1995) .

Table 2.6 Mean number of replants required per plot in autumn, on-off sheep grazing to a 2-3 cm height (RG) or continuous sheep grazing to a 1-2 cm height (CG) (MacFarlane and Sheath 1990)

	1982 replants		1983 replants	
Cultivar	RG	CG	RG	CG
Woogenellup	4.3	6.8	4.7	7.3
Mt Barker	1.3	2.5	2	7

Under continuous closed grazing management at Whatawhata hill country the late flowering cultivars 'Mt Barker', 'Howard' and 'Tallarook' consisted of the highest herbage yield, producing seedling numbers in June of 200 seedlings/m^2. However under rotational grazing conditions early to midseason flowering plants including 'Seaton Park' and 'Clare' and some late flowering plants 'Trikkala' and 'Nangeela' out performed 'Mt Barker', 'Howard' and 'Tallarook'. 'Mt Barker' and 'Tallarook' being small leaved, prostrate runners, with a dense crown can survive under close continuous grazing. However many other cultivars including 'Clare', 'Woogenellup', 'Seaton Park' and 'Trikkala' consist of open habits, with long internodes, low populations of large leaves on long petioles and runners that do not huge the ground. These types are unable to survive closed continuous grazing and are therefore best suited for rotational grazing (Smetham 2003b).

In relation to sub clovers ability to regenerate throughout New Zealand and under a range of management practises, there is currently minimal information available. The information that is available is based largely around older cultivars, which some are not available anymore. From this more research is required on current cultivars and how they withstand a range of management practises.

3 Method and Materials

3.1 Site

An experiment to evaluate 11 sub clover cultivars established with cocksfoot was set up at Lincoln University (43°38′ S, 172°27′ E, 11 m a.s.l.) on Templeton silt loam soil. The site had previously been under an annual cropping regime for six years. Annual rainfall for the long term mean sat at 632mm (1975 – 2013), with a range in monthly air temperatures from 6.1°C in July to 16.6°C in January (Table 3.1). The weather data was provided for the Broadfield Meteorological Station on Boundary Road (Latitude: -43.62622, Longitude: 172.4704), station number 17603.

Table 3.1: Monthly rainfalls and mean air temperatures at Broadfield, Canterbury from January 2014 till September 2015. Long term means are calculated for the period 1975 to 2014 (39 years). (Downloaded from NIWA CliFlo Database 14/9/2015. Station number: 17603)

	Rainfall (mm)			Mean Air Temperature (°C)		
Month	**2014**	**2015**	**LTM**	**2014**	**2015**	**LTM**
Jan	11.4	8	45	15.6	17.7	16.6
Feb	53.5	19	42	16.2	16.2	16.2
Mar	121.7	38.5	51	13.8	15.4	14.7
Apr	160.6	79.4	51	12.4	13.2	12
May	46.3	6.4	58	10	9.9	9.2
Jun	43.4	75.2	64	8.2	7.1	6.5
Jul	48.3	37.3	61	7	6.5	6.1
Aug	13.7	31.2	65	7.5	7	7.5
Sep	25	17.8	40	9.6	7.5	9.4
Oct	19.7		53	11.5		11.3
Nov	48.2		50	13.5		13
Dec	22.1		53	15.2		15.1
Annual	555		632			11.4

3.2 Design

Five replicates of a randomised block design were sown on 14 March 2014, consisting of 11 cultivars of sub clover;' Campeda', 'Whatawhata', 'Napier', 'Narrikup', 'Mt Barker', 'Woogenellup', 'Rosabrook', 'Antas', 'Leura' and 'Denmark', 'Rossi' red clover and 'Nomad' white clover. Plots were 11 m × 6.3 m. A further 11*2.1m cultivar was included in 2014 of sub clover 'Monti', in the western side land of the experiment, due to its early flowering date than the other sub clover species. If this was mixed in with the other cultivars it would have been severally disadvantage by the September grazing, as it would be in full flower leading to a difficulty during regeneration. Seed weight, sowing rate and germination was all measured prior to the experiment and is shown in Table 3.2.

Cultivars were selected in order to test a range of subspecies, flowering times, burr burial and hardseededness, under a New Zealand climate. Older cultivars including ‘Mt Barker’ and ‘Woogenellup’ which were initially used in New Zealand were tested against more modern cultivars including ‘Narrikup’, ‘Monti’ and ‘Rosabrook’ which are not currently commonly sown throughout New Zealand. These cultivars were selected in order to make more reliable recommendations on which cultivars should be sown today, either on their own or as mixes.

3.3 Management

Heavy rain from mid-March to April 2014 (120.4 mm and 139.0 mm) created wet conditions throughout the winter (Table 3.1) preventing herbicide application to control vigorous twin cress (*Lepidium didymum*) growth after sowing. Yield and botanical composition were determined from two 0.2 m^2 quadrats cut to 3 cm residual height on 23 September 2014, followed by sheep grazing to a residual biomass of approximately 1000 kg DM/ha.

Plots were cut in order to remove cocksfoot seed heads from the area in December and the plots were grazed twice by ewes during summer to reduce cocksfoot competition. Rainfall at Lincoln totalled 555 mm in 2014 (1 Jan–31 Dec). In 2015 (1 Jan–30 Jun) rainfall was 227 mm compared with the long term mean for that period (Jan– Jun) of 307 mm. Thus, to ensure sub clover germination occurred at the “normal” time, 25 mm of irrigation was applied with an Ocmus gun irrigator on 10 March 2015.

Due to the visible signs of insect damage beginning to occur which could lead to a data effect, 200 sheep were placed on the plots in order to apply the “hoof and tooth” treatment on May 7th. Sheep were removed on May 11th. Per-pasture measurements were taken, repeating the post-grazing measurements following grazing

Table 3.2: Initial establishment data for subterranean clover plots sown at Lincoln University on 10th March 2014; includes initial 1000 seed weight, number of seeds sown/m^2, sowing rates adjusted for germination and germination. All data was provided by Richard Lucas

Cultivar	1000SW (mg)	Seed/m2	Sowing Rate (kg/ha)	Reps	Germination (%)
Antas	8.7	115.4	10	5	98
Campeda	7.0	200.0	14	5	72
Denmark	6.7	150.0	10	5	81
Leura	7.3	163.6	12	5	77
Mt Barker	9.3	214.3	20	5	41
Napier	11.0	90.9	10	5	96
Narrikup	9.7	103.4	10	5	96
Monti	9.3	107.1	10	10	-
Rosabrook	8.3	120.0	10	5	85
Whatawhata	6.3	189.5	12	5	79
Woogenellup	9.7	103.4	10	5	98

3.4 Measurements

Initial measurements were taken on September 23rd and November 20th 2014, by Richard Lucas to determine yield and botanical composition (weed, sub clover and cocksfoot). Samples from each plot were harvested from two 0.2 m^2 quadrated, except in Monti which had one quadrat per plot. Samples were then dried for 24hours, separated into components and weighed.

On the 11th of March fifty burrs of sub clover were collected from the soil of each plot and were harvested and cleaned by hand, to avoid damage of the seed coat. Three reps of 100 seeds were then selected at random and weighed to calculate the 1000 seed weights. Three replicates of 50 seeds in total were dusted then with thiram, two on the 18th of March and one on the 23rd of March. Placed on wetted germinating paper in an air tight container and germinated in an incubator at 15°C.

Germinated seeds were counted and removed twice daily (8-9 am and 4-5 pm) until germination ceased. Germination was defined to have occurred when the length of the radicle was equal to that of the imbibed seed. Cotyledon emergence was also noted and defined as the presence of two visible healthy cotyledons, in order to assume that the plant was healthy and germination was successful.

Once the rate of germination stopped (5 days without change) seeds were left for 2 weeks in order to define what percentage of the remaining seeds where hard. This was defined by pressing on the individual seeds, if the seed did not flatten or burst it was then defined as hard. Dead seeds were also counted and defined as the imbibing of water with no germination, where pushed on with a fine sharp object would collapse and milky white paste would emerge.

Two 0.1m^2 quadrates were then marked out in each plot at random (excluding Monti which consisted of 1 quadrate marked per plot), using small wooden standards in order to count emergence and population counts over the next few months. From the 23rd of March, following both irrigation and a series of rain events, emergence and plant development began to be monitored two to three times weekly. Emergence was defined as the appearance of two healthy opened cotyledons, with population based on the survival of these seedlings.

Five individual seedlings where then marked using coloured wires within the quadrat, given that there were a healthy seedlings, in order to monitor leaf development of cultivars. The development of the spade leaf was defined as the first leaf on the seedling for simplicity and each individual leaflet from this was counted between two to three times per week. In the case where plots did not contain any healthy seedlings in the marked quadrat area plants were not marked. Likewise following the death of an emerged seedling plants were not remarked.

Plant development measurements were taken up until the 5th of May were marked plants where then harvested slightly below the ground surface, along with a final population count. Where there were fewer than five or no marked plants within a plot, clovers were harvested from around the quadrat in order to have a complete data set (consisting of 50 plants per cultivar). Harvested clovers where placed in a fridge for 24 hours followed by the measurement of the clovers height (bottom of the stem to the top of stem), leaf counts and dry weights. Plant height was taken from the point it was removed from the ground up until the highest leaf.

On the 6th of May prior to the sheep being placed on the trial (pre-grazing), 10 rising plate meter readings were taken. This was followed by 10 pasture cuts to a residual of 1 cm (½m^{2}quadrat) with five pasture meter readings were taken at random. Samples were dried for 48hours and weighed to determine the pasture mass.

3.5 Statistical Analysis

All data was statistically analysed through GenStat using a general one-way ANOVA. In the case of both germination, field and leaf emergences, graphs were produced in order to find a range of dates which showed obvious trends to perform the one-way ANOVA test on. The one-way ANOVA was used to require a significant value along with the LSD and standard errors of the differences between means.

4 Results

4.1 Dry Matter Production in Spring 2014

On September 23rd 2014, sub clover cultivars were harvested, sorted (clover, weeds and cocksfoot), dried and weighed. Harvest total DM yield was lower (P<0.05) for 'Woogenellup', 'Mt Barker', 'Campeda', 'Leura', 'Napier' and 'Rosabrook' at 430 kgDM/ha compared with 'Antas' and 'Narrikup' cultivars at 760 kgDM/ha (Figure 4.1). Sub clover yields were lowest for 'Rosabrook' (150 kgDM/ha) and greatest for 'Antas' (670 kgDM/ha), with 'Antas' yielding more (P<0.001) than any of the other cultivars. The lowest yielding cultivars were 'Mt Barker', 'Denmark', 'Campeda', 'Napier', 'Leura' and 'Rosabrook' (220 kgDM/ha). Cocksfoot yields were greater (P<0.05) with 'Leura' and 'Narrikup' at 150 kgDM/ha compared with an average of 80 kgDM/ha when put with 'Monti', 'Denmark', 'Antas', 'Napier', 'Mt Barker', 'Woogenellup' and 'Rosabrook'. On average the weed yield was 110 kgDM/ha and it was similar between all cultivars.

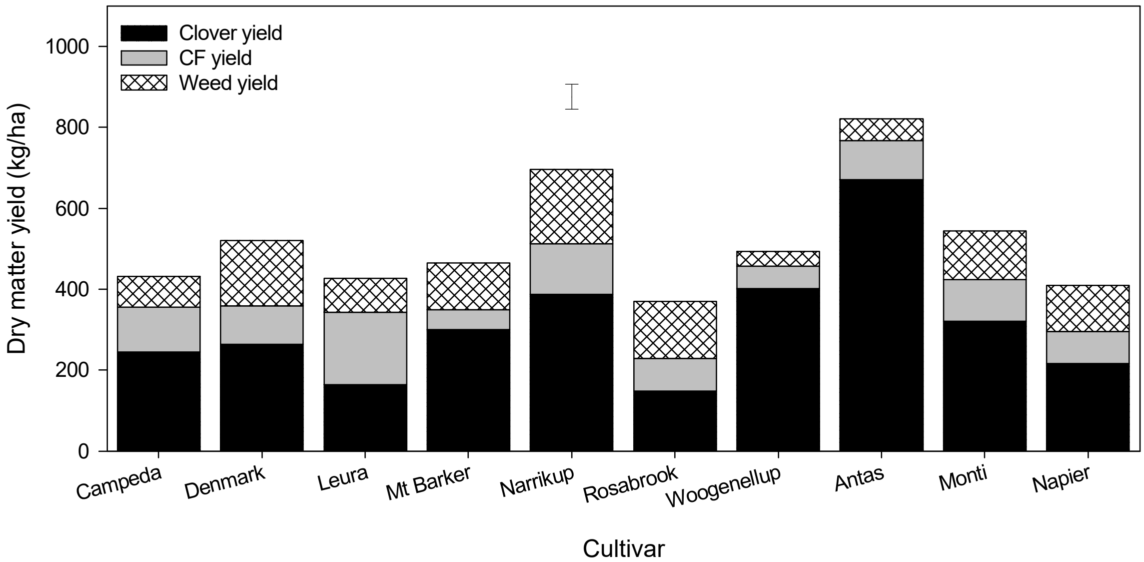

Figure 4.1 Dry matter yield (kg DM/ha) of sub clover cultivars, cocksfoot (CF) and weeds harvested on September 23rd 2014 at Lincoln University, Canterbury. The error bar is the SEM for total dry matter yield.

On November 20th 2014, total DM yield averaged 3520 kgDM/ha and was similar across all cultivars (Figure 4.2). However, sub clover yield was greater for 'Woogenellup', 'Antas' and 'Leura' at an average of 2710 kgDM/ha compared with (P<0.001) 'Denmark', 'Rosabrook', 'Monti' and 'Narrikup' which yielded on average 1470 kgDM/ha. Cocksfoot yield was greatest (P<0.05) in 'Narrikup' and lowest in 'Antas' 160 kgDM/ha. On average weed yielded at 1000 kgDM/ha and accounted for 30% of the total DM yield.

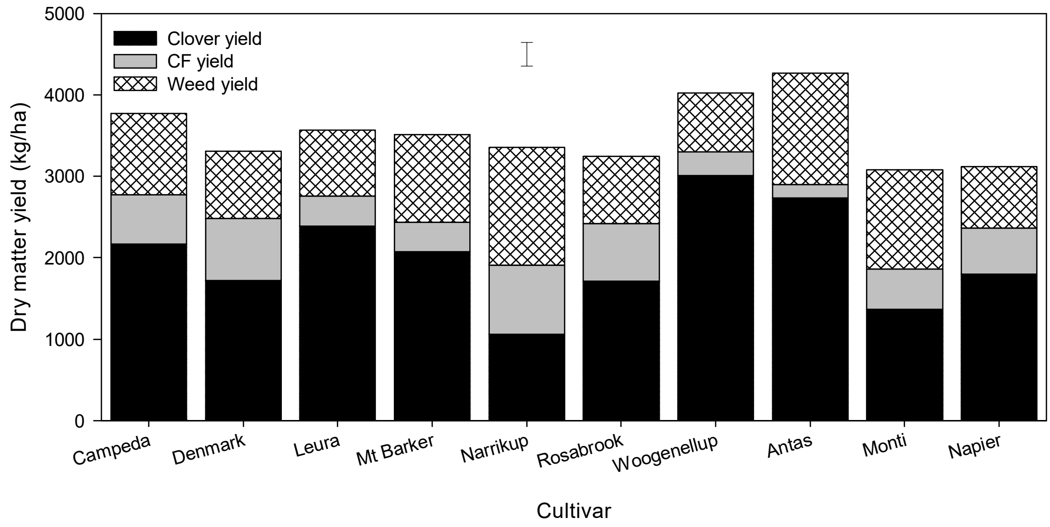

Figure 4.2 Dry matter yields (kg DM/ha) of sub clover cultivars, cocksfoot (CF) and weeds at the November harvest at Lincoln University, Canterbury. The error bar is the SED for total dry matter yield

4.2 Seed weight, germination and hardseededness

From seeds collected on March 11th, 1000 seed weight (TSW) was calculated for each cultivar (Figure 4.3). TSW was lower ($P<0.001$) for 'Leura' at 0.56 g compared to 'Monti' (0.83g), 'Woogenellup' (0.83g), 'Narrikup' (0.80g), 'Whatawhata' (0.80g) and 'Mt Barker' (0.80g). 'Napier' (0.78g), 'Denmark' (0.75g), 'Campeda' (0.68g), 'Rosabrook' (0.64g), and 'Antas' (0.66g) were intermediates in their TSW.

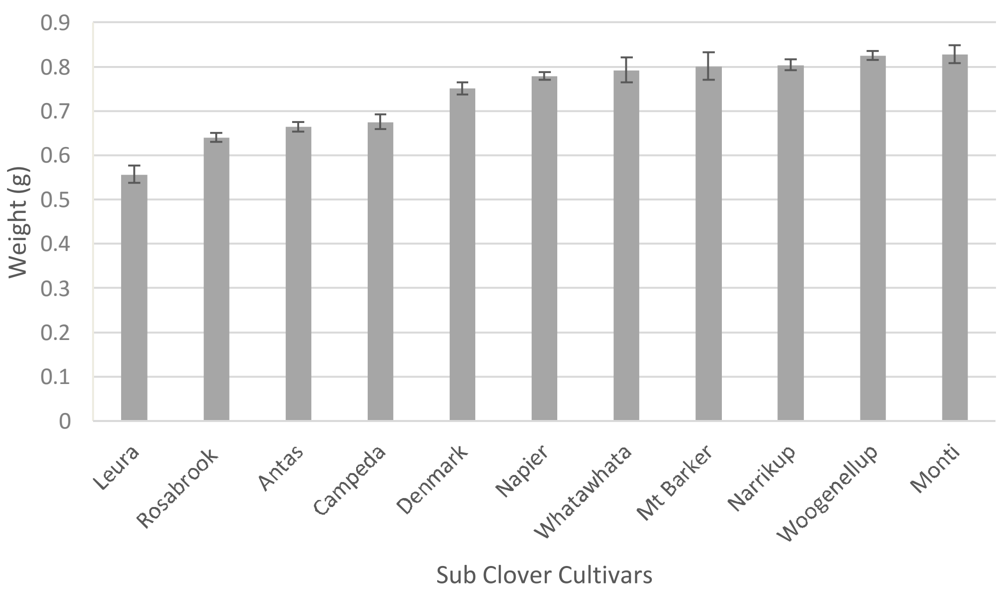

Figure 4.3 Thousand seed weight of seeds collected from burrs on March 11th, of 11 sub clover cultivars, collected for the Lincoln University experimental plots. The error bars represent standard error of difference and the LSD is 0.07g.

Final germination was found to be similar across all cultivars ranging from 23% for 'Leura' to 39% for 'Woogenellup' (Figure 4.4).

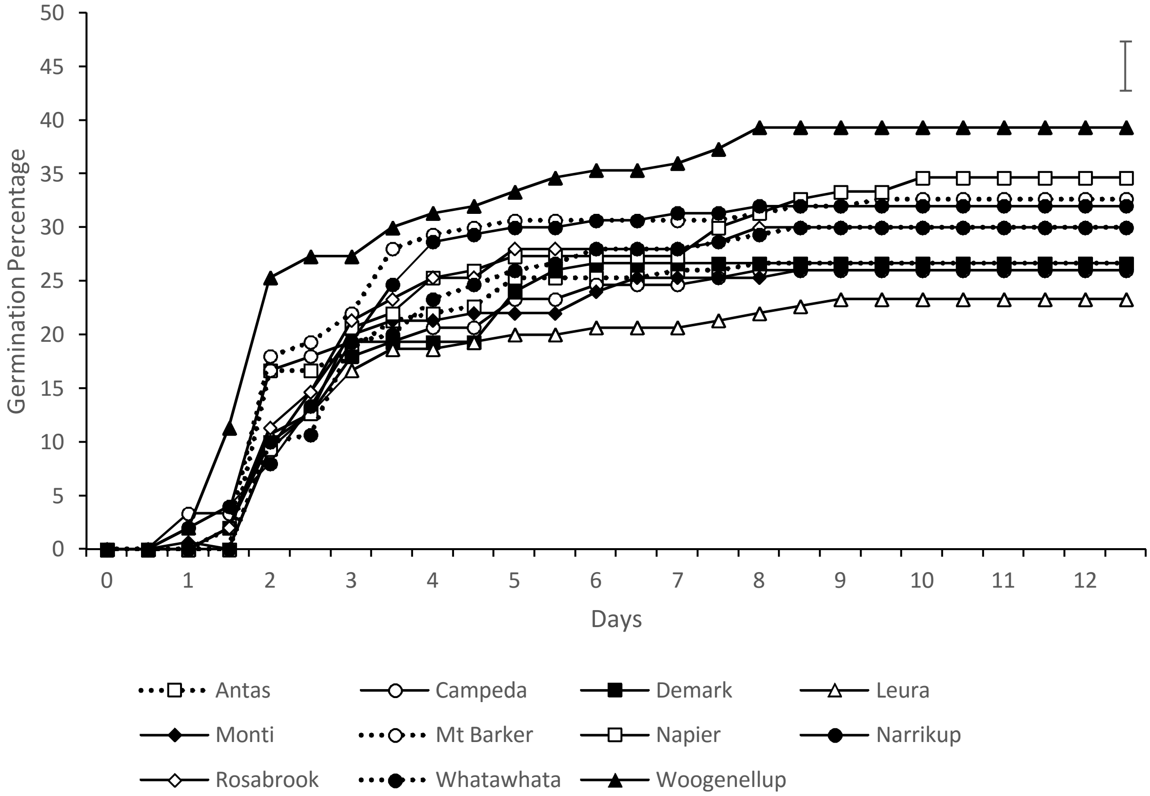

Figure 4.4: Germination percentage of seeds collected from burrs of 11 sub clover cultivars on March 11th 2015 at Lincoln University. Seed were germinated at constant temperature of 15°C. The error bar is standard error of difference between means on day 12.

Most of the seeds that did not germinate were found to be hard (no water imbibed) (Figure 4.4). The remainder were found "dead" as they had imbibed water, not germinated and when applied pressure their structure would collapse. Hardseededness was found to be lowest (P<0.001) in 'Monti' with 34% of seeds remaining hard compared to 'Napier' with only 17%. 'Woogenellup' and 'Antas' also had relatively low levels of hard seed at 19% and 20% respectively.

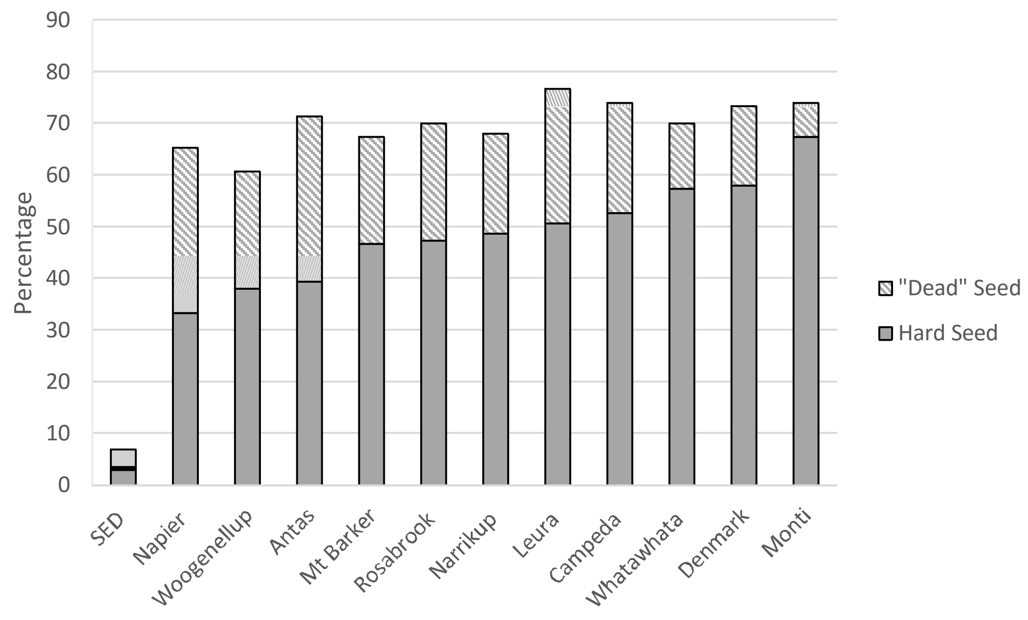

Figure 4.5: Percentage of hard seededness (no water imbibition) and "dead" seed (imbibed but not germinated). From seed samples collected from burrs on March 15th from Lincoln University. Germinated at 15°C until germination had ceased. SED bar represents the standard error if differences between means.

4.3 Seedling Population

Field emergency count data was analysed on the 30th of March, 8th of April and 5th of May. These dates were selected to represent the three main trends on the graph. Seedling population was significantly ($P<0.001$) different for March 30th, April 8th and ($P>0.05$) May 5th (Figure 4.6). When analysed emergence was consistently highest for 'Narrikup' which reached 293 seedlings/m^2 by May 5th. 'Mt Barker', 'Whatawhata' and 'Antas' remained consistently low with an average of 37 seedlings/m^2 by May 5th. Of the intermediate cultivars 'Napier' and 'Campeda' had the higher ($P>0.05$) final emergence averaging at 155 seedlings/m^2 compared to 'Denmark', 'Monti', 'Woogenellup', 'Leura' and 'Rosabrook' averaging at 95 seedlings/m^2.

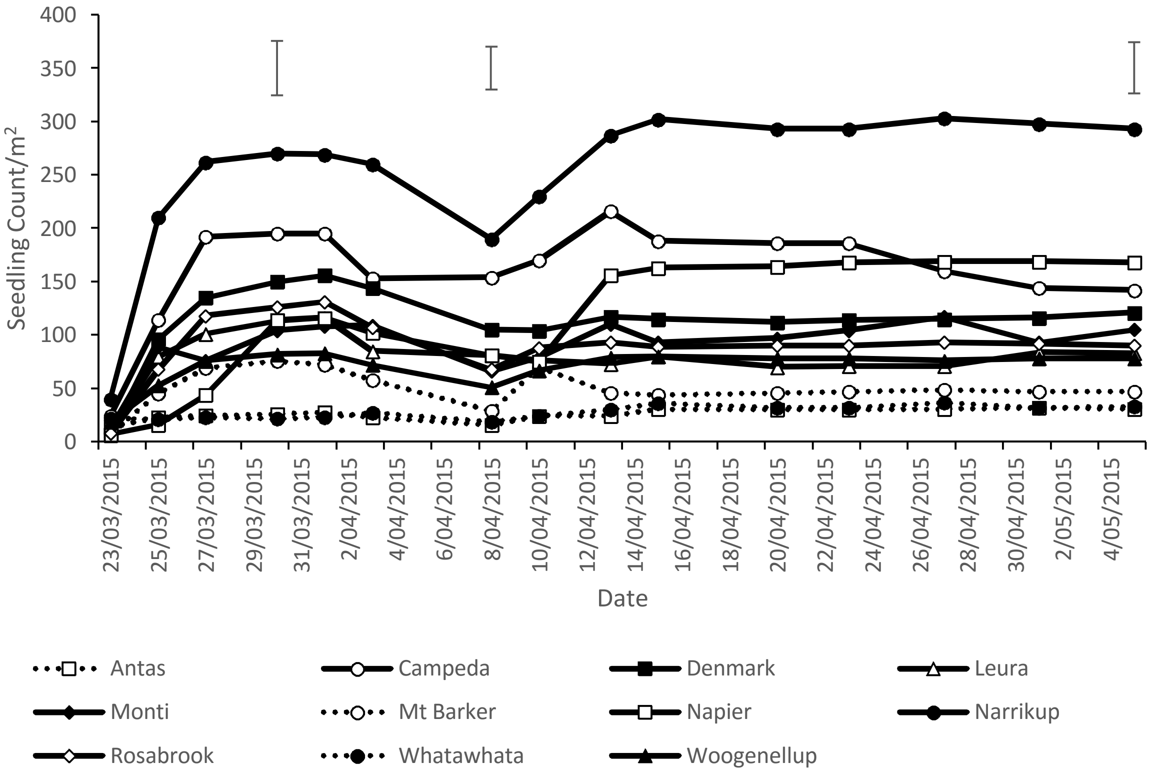

Figure 4.6 Seed population of regenerated seedlings from 23rd of March to 5th of May at Lincoln University. Error bars represent standard error of difference between means for final observations on March 30th, April 8th and May 5th

4.4 Seedling Growth and Development

The number of leaves that formed on each sub clover cultivar seedling following emergence was similar across all 11 cultivars (Figure 4.7). Over 27 days from April 8th to May 5th when leaf appearance was recorded the average leaf appearance for 11 cultivars was 0.15 leaves per day and leaf appearance interval was 7 days per leaf.

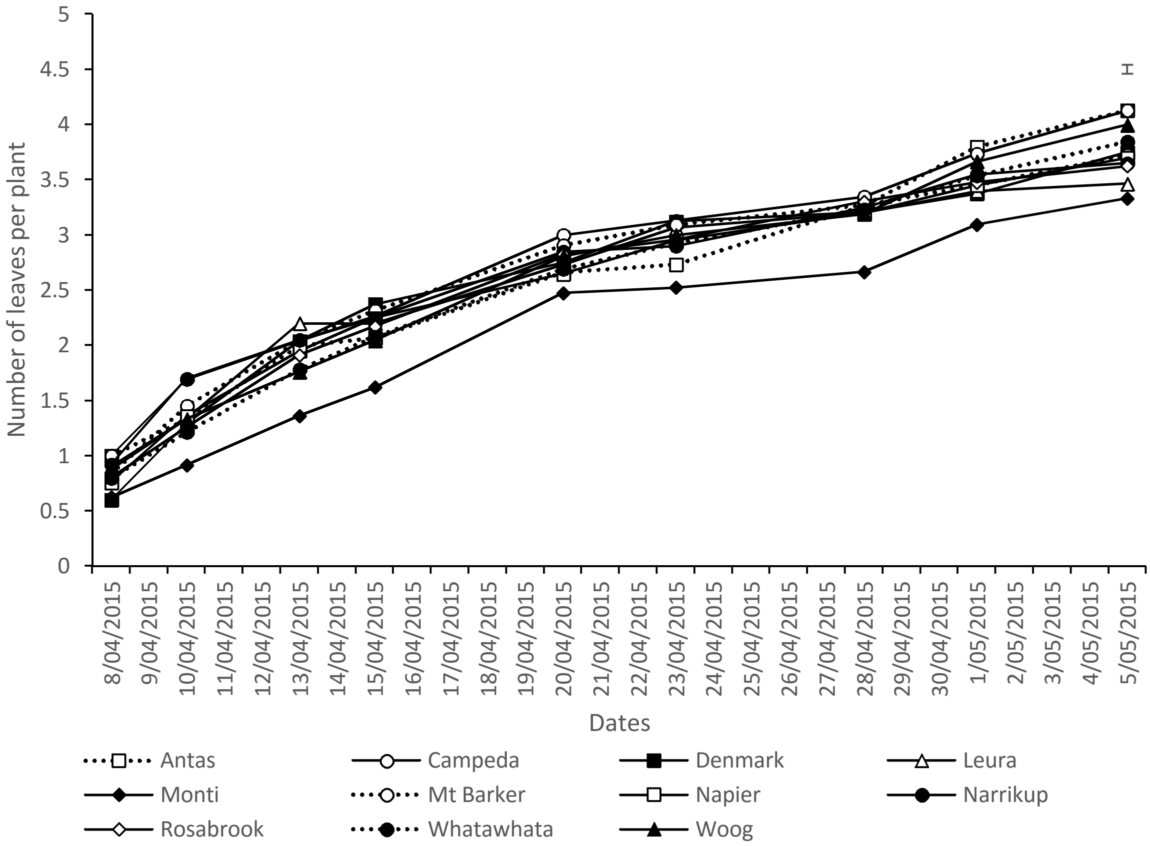

Figure 4.7 Number of leaves formed on individual seedlings of 11 sub clover cultivars from the 8th of April to May 5th at Lincoln University. The error bar is standard error of difference between means on the 5th of May.

The height of sub clover was found to be lowest (P<0.05) in 'Leura' and 'Mt Barker' at 1.2 cm compared to 'Woogenellup' and 'Monti' averaging at 2.9 cm (Figure 4.8). Remaining cultivars were similar in height averaging at 1.9 cm.

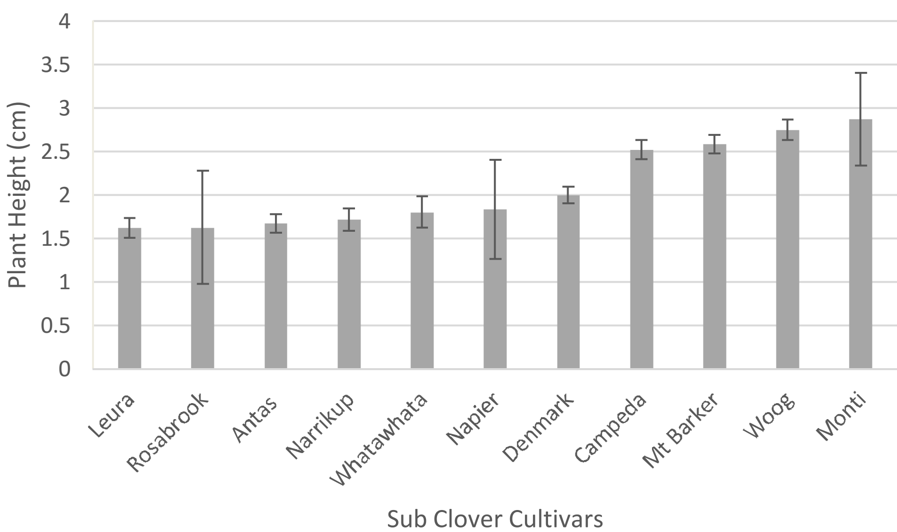

Figure 4.8 Average height in cm of 11 subterranean clover cultivars following harvesting on the 5th May 2015. The error bars represent the standard error of means

Sub clover weight (g) was lowest ($P<0.05$) in 'Mt Barker' at 0.014 g compared to 'Whatawhata' and 'Antas' at 0.02 g (Figure 4.9). All other cultivars were found to be similar weights at 0.018 g.

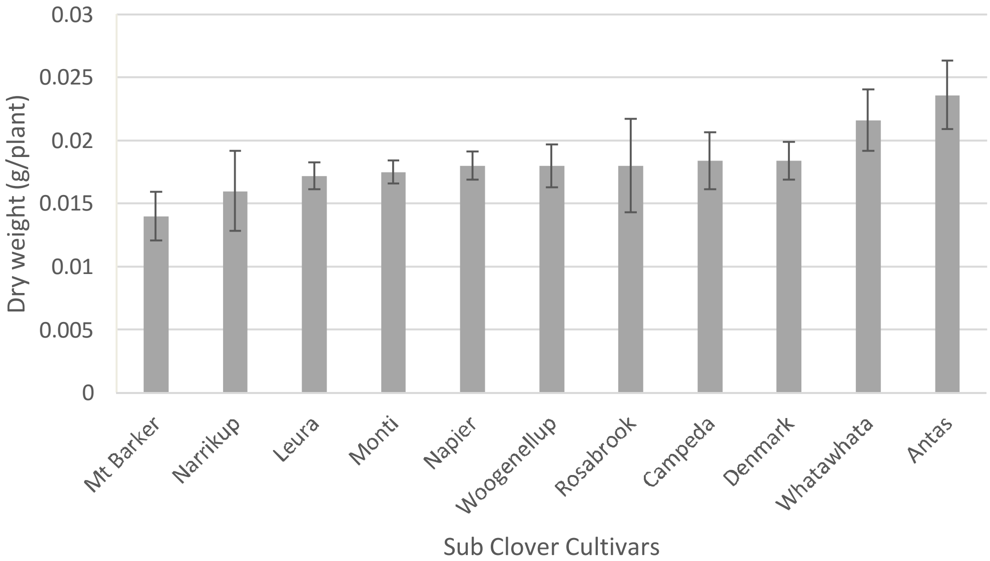

Figure 4.9 Average dry weight in grams of 11 subterranean clover cultivars for individual plants, following harvest on May 5th. The error bars represent the standard error of means.

All pasture cuts were found to be similar with the average dry weight averaging 32g (Figure 4.11).

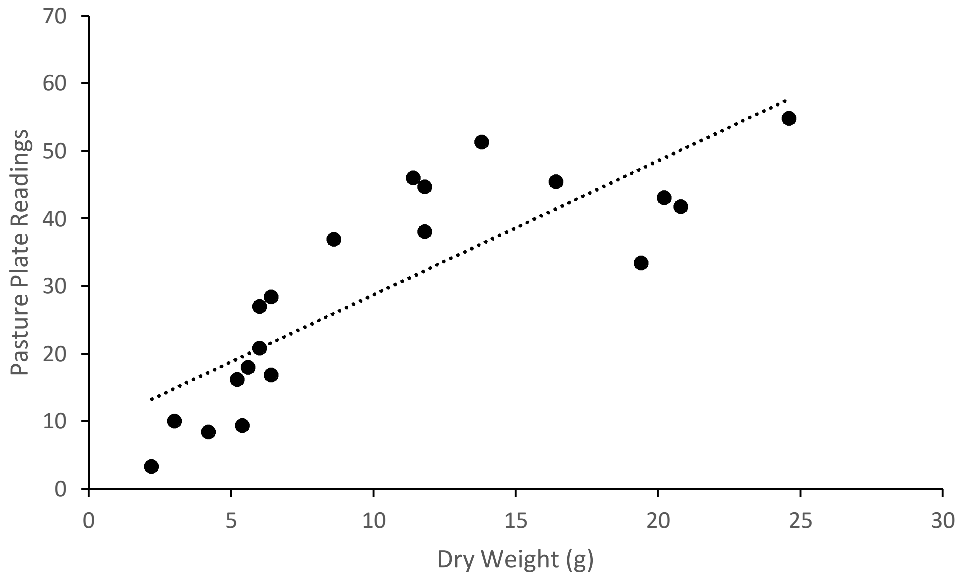

Figure 4.10 Pasture plate readings and dry weight of 20 randomly selected sights harvested pre grazing (1) on May 5th and post grazing (2) on March 7th, using a 0.5m^2 quadrat. R^2 value of 0.6905

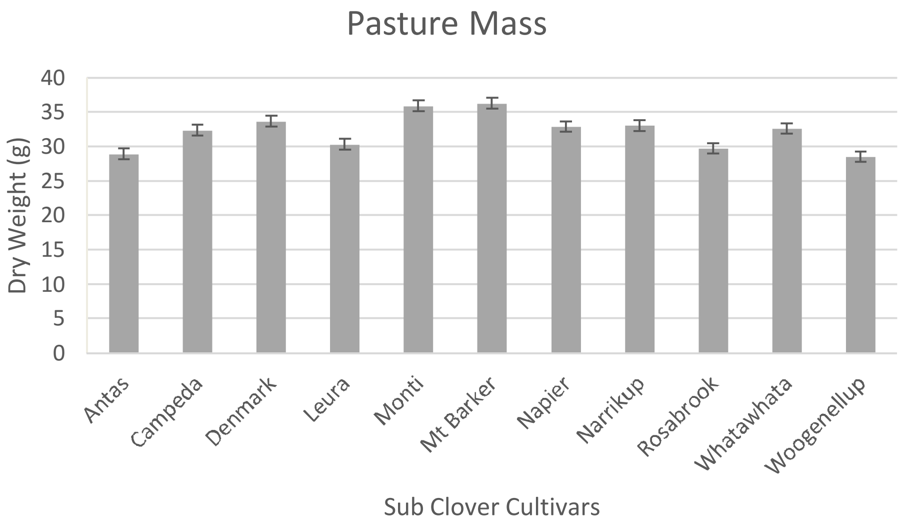

Figure 4.11 Average pasture mass taken across 11 different subterranean cultivars. Pasture mass estimate derived from Figure 4.9 equation (y = 1.9803 * x + 9.0309), used to calibrate plate meter measurements. Each cultivar consisted of two quadrat cuts from each plot, therefore 10 readings for each cultivar in total.

On September 30th 'Napier' and 'Woogenellup' had the highest (P<0.001) sub clover cover of all cultivars at 7.0 (Figure 4.12). 'Mt Barker' and 'Denmark' were found to be higher (P<0.05) averaging at 4.0 then the intermediate cultivars 'Campeda', 'Monti', 'Antas', 'Rosabrook' and 'Whatawhata' averaging at 2.0. 'Leura' and 'Napier' consisted of the lowest cover at 0.8.

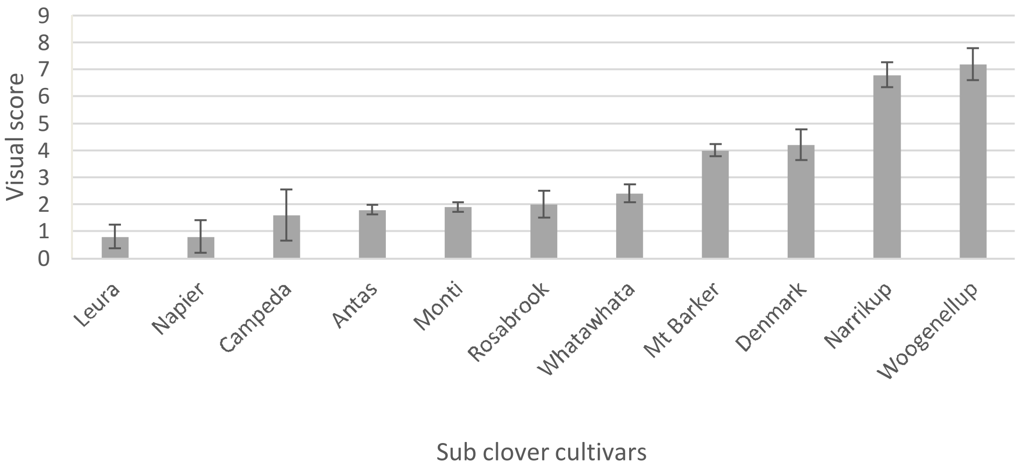

Figure 4.12 Final visual score of 11 subterranean clover cultivar, ranging from 0 – no visible sub clover to 10 – 25% sub clover cover. Visual scores were taken on 30/09/2015. The error bars represent the standard error of means.

5 Discussion

5.1 Sub Clovers First Year Performance

In September 2014 (Figure 4.1), DM production varied significantly between cultivars, with 'Antas' and 'Narrikup' sitting at the highest (760 kgDM/ha). 'Antas' showed the highest potential of the sub clover cultivars in the early spring (670 kgDM/ha), with the general trend on the initial DM showing later flowering cultivars including 'Leura', 'Denmark' and 'Rosabrook' to be lower yielding in September. The current recommendation in New Zealand however is for late flowering cultivars due to their ability to regenerate, unless under sever moisture stress (Chapman 1992). By November cultivars did not vary in DM yields showing the potential for later flowering cultivars to perform, with the highest clover component in 'Woogenellup', 'Antas' and 'Leura'.

Cocksfoot competition appeared to play a significant role with some of the later flowering cultivars not being able to produce the highest yields. Although sub clover has previously been found to be compatible with cocksfoot (Brown et al. 2006) other studies have also shown that cocksfoot can out compete sub clover cultivars negatively impacting on their performance Dear and Cocks (1997). 'Leura' was the only late flowering cultivar to overcome the spring cocksfoot and weed competition. 'Denmark' and 'Rosabrook' however consisted of the lowest sub clover yields in the November harvest (1470 kgDM/ha).

Futhermore it would have been expected that the two *yanninicum* sub clover cultivars 'Monti' and 'Napier' would have stood out throughout the 2014 data set. *Yanninicum* has adapted to survive and outperform other subspecies under wet conditions (Costello & Costello 2013). As conditions were wetter from March through to July 2014 compared to average (420 mm, 2014. 285 mm, average) other subspecies which have not adapted to water logged conditions may have been at a disadvantage. This however was not the case and there is no evidence to conclude that the *brachycalycinum* or *subterraneum* were disadvantaged in any way in the wetter conditions.

5.2 Germination

Germination of sub clover seed can be effected by a range of factors including hardseededness (Nichols *et al.* 2013), climate (Smetham 2003a) and dormancy (Black 1956). With this in mind it has been found that cultivars vary in their germination potential within the field (Nichols *et al.* 2013). However under the Lincoln University lab germination cultivars were all found to be similar (P>0.05). Germination of the 11 sub clovers was on average poor with the highest germination of 39% ('Woogenellup') of the total seed.

The issue with germination could be related to both the New Zealand climate and its effect on hardseedness. In order for the hard seed coat of sub clover to break down efficiently it requires a fluctuation in temperature of 15 - 30^{0}C (Smethan & Ying 1991). Hardseededness remained high throughout the experiment ranging from 33% in 'Napier' to 67% in 'Monti' ($P>0.001$). This could be due to the Canterbury climate. During the summer months in 2014/2015 air temperature did not reach or exceed 30°C except for two days in January and February, averaging at 23.4°C and 23°C respectively, with soil temperatures at 10mm never exceeded 24.7°C. Without the high fluctuation in temperatures, the breakdown of the hard seed coat is slowed, preventing water from imbibing and effecting germination (Smethan & Ying 1991), which would explain the high levels of hardseededness along with poor overall germination.

Another factor of low germination could be related to the level of dead seeds. Although this was not significant between cultivars, it was expected that seeds would either germinate or remain hard. Dead seed levels ranged from 8% for 'Monti' to 32% in 'Antas' and 'Napier'. Smetham (2003a) states that in circumstances were moisture stress occurs during the maturity of the seed, the suberin layer can be thinner and in some cases even discontinuous. Therefore in cases were moisture deficit occurs soon after flowering, leading to a short flowering period the seed can lack in the hard seededness which can result in seed lost when imbibing moisture. Furthermore Hagon (1971) found that temperature variation under a cooler climate can soften the seed because of the expansion and contraction of the tissue, leading to rupture and chemical degradation of the testa. This then allows moisture to enter the seed but will result in a swollen fleshy addition to part of the ovule and can lead to unviable seed (Smetham 2003a).

5.3 Hardseededness

Hardseededness between cultivars was found to vary significantly ($P < 0.001$) throughout the lab germination. This was expected due to published data showing a range of hardseededness levels in cultivars used throughout this experiment; 5/10 (Campeda, Napier and Rosabrook) to a 1/10 (Mt Barker) (Nichols *et al.* 2013). However the highest level of hardseededness was found in 'Monti' (67%), 'Denmark' (58%) and 'Whatawhata' (57%) and the lowest in 'Napier' (33%), Woogenellup (38%) and 'Antas' (39%) which differs from the published data (Table 2.1), showing no relationship between the two data sets (R^2: 0.0453). This shows that although cultivars do vary in their hardseededness, Australian data should not be used to make recommendations throughout New Zealand.

Flowering date may also have an effect on the level of hardseededness of a cultivar. With previous data examining flowering dates, early flowering cultivars have been found to exhibit higher levels of hard seededness following maturity than late flowering cultivars (Smetham & Ying 1991).

This is presented in the data under this study with the lowest levels of hardseededness found in ‘Napier’, ‘Woogenellup’ and ‘Antas’ which are all later flowering cultivars (over 130 days), whereas ‘Monti’ is the earliest flowering cultivar in this experiment (110 days) and exhibits significantly higher levels of hardseededness (P>0.001).

5.4 Natural regeneration

An essential feature when using sub clover is its ability to persist within a mixed population. Figure 4.5 shows the ability of 11 sub clovers during their first natural regeneration in the autumn 2015. By May 5th 2015 cultivars varied significantly (P<0.05) from Antas at 31 seedlings/m^2 to Narrikup at 293 seedlings/m^2. Narrikup was found to have up to 92% higher seedling population then the other cultivars throughout the complete population counts. It is notable that this cultivar consists of a rating of burr burial of 7/9 and a medium level of hardseededness at 48% (Figure 4.4). Widely sown cultivars including Mt Barker in contrast had low seedling populations (47 seedlings/m^2 on May 5th). Although ‘Mt Barker’ was found to have similar levels of hard seed at 46%, it consist of a low burr burial ability of 3/9 (Nichols *et al*. 2003) and burr burial may have led to these results.

With the highest performing cultivar displaying a high burial rate it shows how significant this trait may be under the New Zealand climate. With some sub clover cultivars having the ability to develop its seeds beneath the soil surfaces, this can lead to increased protection from unfavourable aerial environments. Sub clovers ability to bury its seed will lead to protection from the initial development stages until the seed is fully matured (Collins *et al.* 1976). Burr burial varies significantly between cultivars (Nichols *et al*. 2013). Results from this study reinforce the current information available with ‘Antas’ consisting of the lowest burr burial (1/9) and the poorest field emergence. While ‘Narrikup’, ‘Campeda’ and ‘Napier all consist of high burial ratings (7/9 and 6/9) and comprised of the highest seedling emergence and initial population.

From the first emergence there was a steady increase in populations for majority of the cultivars following the first autumn rains. However from the 3rd of April to the 8th of April there was a sudden decrease in seedling population, with the most obvious of these in ‘Narrikup’ which lost 27% of its population (Figure 4.5). The most probable cause of this could be attributed to ‘false strike’, which in previous studies was found to be a significant issue throughout New Zealand (Dodd *et al* 1995; Sheath & MacFarlane 1990). False strikes occur under the New Zealand climate due to a cultivars tolerance of summer rainfalls. In cases where there are regular summer rainfalls losses of up to 55% of a sub clovers population have been found to occur each year (Dodd *et al.* 1995). However following a further serious of rain events cultivars which were affected by this ‘false strike’ appeared to on average recover their populations.

An interesting point to note from this experiment is related to flowering. Currently late flowering cultivars are recommended throughout the New Zealand climate, unless the area is significantly affected by early drought (Hoglund 1990). This recommendation comes from the late flowering plants having a greater opportunity to exploit favourable spring growing conditions, resulting in better development of the seed (Chapman 1992). However from this experiment there is no distinct advantage between early, mid or late flowering cultivars. 'Narrikup' which sat at the highest population count throughout the experiment has 127 days till flowering, whereas Antas which remained the lowest has 138 days until flowering (293 seedlings/m^2 verse 31 seedlings/m^2 on May 5th). Even the earliest flowering cultivar 'Monti' with 110 days until flowering did not appear to be disadvantaged, sitting in the intermediate at 105 seedlings/m^2 on May 5th.

5.4 Plant development

Overall cultivars early leaf development was found to statistically similar (P>0.05) following emergence (Figure 4.6). This is what would have been expected as initial morphological development following germination of all pasture legumes does not vary significantly (Thomas 2003). Murray (2012) also found this with no difference between initial leaf developments between clover species or within the sub clover species 'Rosabrook' and 'Napier'. Upon the emergence of sub clovers cotyledons they serve no further storage role, with growth becoming highly dependent on the cotyledon area and the root structure (Rossiter 1992). This therefore means that the advantages a seedling cultivars may have initially had from its stored energy (seed), due to seed size and/or burr burial are no longer occurring. With no measurements of initial seedling size throughout cultivars however it is just an assumption that there was little variation in seedling size between cultivars following emergence.

Plant height was then measured from the plants harvested on the 5th of May and found to vary significantly between cultivars (P>0.05). Although there is currently little information on height between cultivars, sub clover in general has found to be a short clover species of up to 12cm (Hollander *et al.* 2007). Lucas 2015 identifies some properties of sub clover stating that both 'Antas' and 'Woogenellup' have long petioles which could lead to taller plants. This is shown in 'Woogenellup' which is, along with 'Monti', the tallest cultivars (2.7cm and 2.78cm respectively), but not in 'Antas' which on a whole performed poorly throughout this experiment. Cocksfoot competition was initially believed to lead to the height of 'Monti' as under grass competition clovers will extend their petioles to compete for light (Tow & Lazenby 2002). However although pasture appeared thicker and taller through the 'Monti' plots this was not the case follow pasture plate measurements (Figure 4.10).

Following height measurements of sub clover, dry weight was also measured (Figure 4.8) and found to vary significantly ($P<0.05$). Although there was a very slight trend between sub clovers height and weight (R^2: 0.1306), shown in Figure A.2, plants dry weight is not based on the plant height. This shows that although a sub clover cultivars may not be tall they may have larger leaf areas an example being 'Antas' which had one of the shortest stems (1.67 cm) but was the heaviest (0.0236 g) and has been identified to have large leaves (Lucas 2015). Dry weight of young seedlings has also been positively attributed to initial seed size (Moot *et al.* 2000). However under this experiment, initial seed weight at sowing was found to have a weak relationship (R^2: 0.1191) but the interesting point here was that it was negative relationship (Figure A.3).

5.6 Cultivar Evaluation

'Narrikup' which consistently performed well throughout emergence and population counts, remained one of the best performing cultivars with a visual score of 6.8 (roughly 15% clover content). This shows that with its high potential of burr burial (7/9) (Nicolas *et al.* 2013) this protection mechanism from the surrounding environment (Collins *et al.* 1976) has led to a significant advantage in this cultivars ability to persist and survive under the New Zealand climate. Another point worth mentioning about 'Narrikup' is that when exposed to frost damage (Figure A.1) 83% of its seedlings were damaged, however it managed to recover and still remain prominent in its sub clover cover, which shows huge potential under the New Zealand climate.

Both later flowering cultivars 'Mt Barker' and 'Woogenellup' which are currently widely used throughout New Zealand (Black 1957), began with a relatively poor population (47 seedlings/m^2 and 78 seedlings/m^2). However in the final visual score, 'Woogenellup' consisted of the highest clover cover (7.2), with Mt Barker also performing well in comparison to many of the intermediate cultivars (4.0). Another late flowering cultivar 'Denmark' also performed well in the final cover, scoring 4.2. With nationwide evaluations finding that the late cultivars are the most persistent under the New Zealand climate due to their ability to exploit favourable spring growing conditions (Chapman 1992), these results are not surprising.

'Antas' is another interesting cultivar which should be taken note of. 'Antas' comes from the sub specie *brachycalycinum* and is a late flowering type. This cultivar showed huge potential in its first year reaching 2710 kgDM/ha of sub clover in its pasture mix, along with the highest overall yield in September (760 kgDM/ha). However it was unable to perform in its second year, due to its inability to regenerate. This can be attributed to its sub specie *brachycalycinum*, which does not actively bury its burrs. The interesting point to make here is that 'Antas' could be extremely useful under the New Zealand climate as an annual pasture mix. It could be beneficial in years were other legumes are struggling to perform but are likely to come back in the following year and a quick fix is required.

In 2014, 'Leura', a late flowering cultivar was attributed to being able to overcome cocksfoot and weed competition in the spring months, but did not perform in the following year. Leura began poorly in the 2015 season (83 seedlings/m^2) and got worse as the season progressed, with a visual score below 1 in spring. The reason behind this poor performance is puzzling as 'Leura' has a relatively high burial score (5/9) (Nichols *et al.* 2013) and was an intermediate in its level of hard seed (Figure 4.5). All of the characteristics of this cultivar would suggest a strong performance under the New Zealand climate (Chapman 1992; Collins *et al.* 1976; Smetham and Ying 1991; Sheath and Richardson 1983), but under the 2015 season 'Leura' did not perform up to expectations.

'Campeda' is another cultivar of interest throughout this experiment. Initially this cultivar began strongly reaching 216 seedlings/m^2 by the 13th of April, but following this seedling population then began to drop (142 seedlings/m^2 on May 5th). Although throughout the population counts it remained in the top 3 performing cultivars by spring this cultivars performance had deteriorated (visual score of 1.6). This may have been due to this sub clover cultivar being less able to compete in a cocksfoot pasture compared to the rest. Dear and Cocks (1997) found that of seedlings which emerged in March only 1% of these survived in a cocksfoot pasture going into the spring due to competition for water and nutrients compared to 78% in a pure sub clover pasture. Although sub clover has previously been deemed compatible with cocksfoot (Brown *et al.* 2006), these results show that this is cultivar dependent.

'Napier', a cultivar released in 2000 (Nichols *et al.* 2006) was another sub clover which was expected to perform well under Canterbury conditions. 'Napier' is an adaption from a former cultivar 'Larisa' of the *yanninicum* species, which has previously performed well under New Zealand conditions, particularly wet conditions. Under an experiment at Kaikohe 'Larisa' outperformed 'Mt Barker' by 63% which shows the potential of 'Napier' (Sheath *et al.* 1990). 'Napier' was put onto the market as it was found to have greater winter and herbage production, high production of seed reserves and stronger regeneration than the late maturing cultivars 'Larisa' and 'Meteora' (Nichols *et al.* 2006). Even under a previous Lincoln University experiment germination reached 96% under lab conditions (Murray 2012). However in this experiment 'Napier' performed averagely, reaching only 36% germination which could be due to the difference in seed collection, bought seeds verse hand harvested.

'Rosabrook' is one of the newest cultivars on the market released in 2009 and is a crossbred of 'Denmark' (Nichols *et al.* 2013) which showed potential under a New Zealand climate. Although this cultivar did not stand out compared to the cultivars including 'Narrikup', 'Campeda', 'Denmark' and

‘Woogenellup’ it did show the ability to grow following sowing, to regenerate and persist. Initially this cultivar was among the highest yielding in September 2014 (760 kgDM/ha) but performed at an intermediate level in the following season with 90 seedlings/m^2 during natural regeneration and a final clover cover of 5%. Which shows that although this cultivar may not be the ‘best’ throughout this experiment, it does have the capability to survive under New Zealand conditions.

An early flowering cultivar ‘Monti’ was placed in this experiment to cover all ranges. It is believed that under the New Zealand climate early flowering cultivars can perform poorly due to high levels of hardseededness (Smetham & Ying 1991). ‘Monti’ did exhibit large quantities of hardseededness, yet still was able to emerge (105 seedlings/ m^2) and persist (5% final spring clover cover) and was found to be superior then some of the later flowering cultivars including ‘Napier’ and ‘Leura’.

Under this Lincoln University experiment, a new cultivar which has not currently been released was also used. ‘Whatawhata’ is a New Zealand cultivar which has currently not been investigated fully under any New Zealand conditions and is therefore difficult to predict its performance (MacFarlane *et al.* 1990). Throughout this study on a whole the cultivar performed poorly exhibiting high levels of seed hardseedness (56%), low field emergence (27 seedlings/ m^2) and a 5% final clover cover in spring.

5.7 1000 Seed weight

Sub clover is believed to have a large seed size which can allow for quick establishment and rapid early growth when compared to many other perennial legumes (Smetham 1968). Seed weight of these clovers will vary between cultivars and can also be effect through the climate and management of the cultivars (Ates *et al.* 2006). Under the Lincoln University experiment on sub clover cultivars a significant variation (P<0.001) between the 1000 seed weight of individual cultivars was found. Seed weight ranged from 56mg ‘Leura’ to 83mg ‘Woogenellup’. Previous Australian data recorded (Table 2.1) a variation between cultivars and sub species, which would be expected (Nichols *et al.* 2013).

From the experiment at Lincoln University the 1000 seed weight appeared to be highest in the *yanninicum* sub specie (Figure 5.1). Although some of the *subterraneum* cultivars also consisted of some of the highest seed weights (i.e. ‘Woogenellup at 97 mg at sowing and 82 mg following seed set) this sub species had a significantly higher range in its 1000 seed weight; 56 mg (‘Leura’) to 82 mg (‘Woogenellup’) following seed set in 2015. The *brachycalycinum* sub specie ‘Antas’ was again significantly affected by its ability to bury its burrs with a 1000 seed weight at sowing at 87 mg, but reducing to 67 mg.

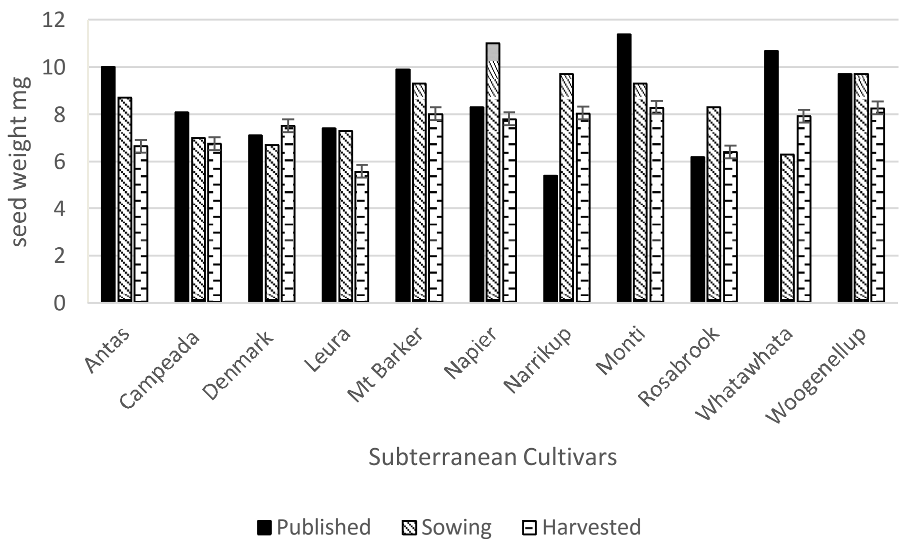

Figure 5.1: 1000 seed weight of 11 subterranean clover cultivar seeds, collected for the Lincoln University experimental plots, all manually harvested and cleaned, compared to published data and the weights initially sown

In relation to seed size, burial in the field not only results in up to a fivefold increase in seeds per burr, but also has a positive increase in the weight during seed produced. In cases were burial has been artificially prevented, seed weight has been found to reduce by 50% of its predicted weight under natural conditions (Smetham 2003a).

The subspecies *subterraneum* and *yanninicum* have the ability to actively bury their burrs. Their peduncle become elongated and thicken allowing for them to actively push their burrs into the ground, becoming anchored by the sterile calyces which form the burr structure. The sub specie *brachycalycinum* is known for having long thin sarmentous peduncles which spread until they find a suitable dark habitat, such as under stones or cracks which have formed in the soil and therefore does not bury its burrs (Bolland & Collins 1986).

In relation to published data, however, variations where found when comparing to the seed weight of the second year crop. Harvested seed weights decreased between 6 – 34% in weight when compared to published data from Australia (Figure 5.1), with the exclusion of Narrikup. Although it is not known why this was caused, it could be linked to both the New Zealand climate in comparison to the Australian or something as simple as the screening process seeds go through before becoming marketable.

Successful seed burial in sub clover is directly related to the spring moisture during flowering (Nicholson 2006). In 2014 the season was on average found to be relatively dry compared to the long term mean. Through September, October and November in 2014 the rainfall (mm) was 38%, 64% and 4% lower than the average long term mean. If burr burial is directly related to the spring conditions then this could have led to the effect on the capability to bury their burrs and therefore could have been a reason for the average reduction in seed size.

5.8 Spring Cover and Grazing

Dry matter was going to be collected in a similar fashion to 2014. However due to the initially poor establishment over the majority of the cultivars this was ruled out as in many cases sub clover wasn't present within many of the plots. Furthermore in June there became an issue with plants dying after they had turned red which was originally unidentified and therefore a record of affected plants was taken (Figure A.1). This was later identified as frost damage which sub clovers have been found to be highly sensitive to (Boswell *et al.* 2003).

Under this experiment grazing was used mainly as a tool to control cocksfoot competition. As the majority of these cultivars consist of open growing habits (which is now selected for in Australia), excluding 'Mt Barker' (Smetham 2003b), grazing was kept to a minimum to avoid damage to the sub clover. However with the discoveries of this experiment, grazing methodology and animal effect may be the next step in promoting the use of sub clovers throughout New Zealand.

5 Conclusion

The aim of this study was to evaluate a range of sub clover cultivars and their ability to regenerate and persist under a New Zealand environment. Currently the sub clover cultivars which are available in New Zealand are Australian cultivars and associated data. However due to the large variations in climates it has been found that this data is not reliable and there is a need for independent New Zealand studies which will allow appropriate recommendations as to what cultivars would persist under a New Zealand climate.

On average cultivars performed poorly in their ability to germinate under lab conditions. From this a large proportion of seeds remained hard and varied significantly between cultivars. Monti was found to have the highest level of hard seed at 67%, while Napier was found to have the lowest at 33%. This shows that under the cooler New Zealand climate some cultivars may be severally jeopardised at the germination stage. However due to the high level of variation between the cultivars it shows selection can be made between cultivars, reducing the negative effect that high levels of hard seed can have on sub clovers performance.

'Narrikup' was found to have the highest level of performance in its ability to regenerate and persist. This mid flowering cultivars presents the importance of seed burial under the New Zealand environment. With its high ability to bury seed burrs (7/9) it was able to develop underground whilst remaining unaffected by the aerial environment. 'Narrikup' was able to emerge quickly and survive, right through till spring, when its performance still remained high with an average of 15% clover content in the clover cocksfoot sward. Although more research would be required before a suggestion can be made to sow purely 'Narrikup' in a pasture mix, it presents the possibility of the new cultivars being able to persist and perform under the New Zealand climate.

Both 'Woogenellup' and 'Mt Barker' began poorly during emergence (47 seedlings/m^2 and 78 seedlings/m^2 by March 5^{th}) but recovered by the final visual score in spring. As spring pastures are essential to dry land farming, this shows that these late flowering, original, cultivars still have their place in the New Zealand pastures. It allows for recommendations including both of these cultivars, to be mixed with a new high performing cultivar such as 'Narrikup'. These results show that these cultivars have huge potential under the New Zealand dryland system, with the next step being looking into their management and how this can benefit the industry.

This data brings to light several queries from previous research including flowering date and how this cause cultivars to vary. Although current recommendations have been based around selecting late flowering cultivars, this research suggest that mid-flowering cultivars will perform just as well and in some cases better. Furthermore it has presented just how differently these cultivars will react under a temperate climate such as New Zealand's. Particularly hardseededness data is something that should be used carefully, as Australian data has proven to be extremely different to hardseededness from those grown under the New Zealand climate.

Overall from this study has shown that a range of sub clover cultivars have real potential under the New Zealand climate along with questioning a lot of the current recommendations which have been based on Australian data. This opens opportunities for new cultivars to be used in pasture mixes, with New Zealand data to back up claims.

Appendix A

Sub clover experiment

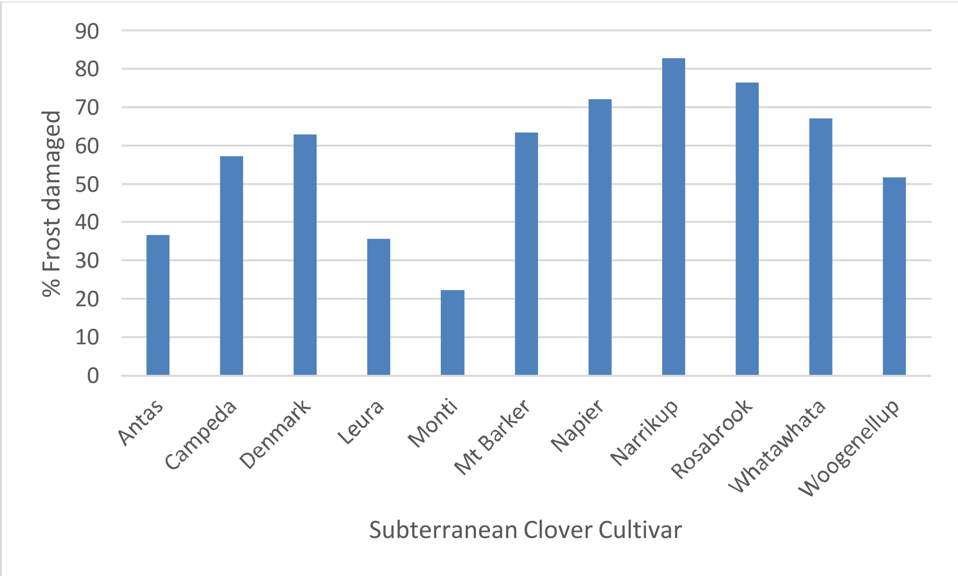

Figure A.1: Percentage of subterranean clover cultivars damaged by frost. Measured in $0.1m^2$ quadrat, with frost damage measured by colour (red plants were deemed as damaged), 15/06/2015

Table A.1: Subterranean clover cultivars identification features (Lucas 2015)

Cultivar	Identification
Antas	• Slightly hairy or hairless • Green stipules at petiole bases have red stripes • Strong leaf mark • Large leaves and long petioles • Black seed
Campeda	• Hairless runners • Red striped stipules • Faint orange band on flower
Denmark	• Hairless runners, petioles and peduncles • Light leaf mark • Small leaves.
Leura	• Hairy runners • Stipules are green
Monti	• Hairless runners and petioles • Green stipule with red stripes • Brown flecks in late autumn/winter on leaves
Mt Barker	• Very hairy runners and red stipules • Brown flecks on leaves in winter • Red band on flower tubes
Napier	• Creamy white seed
Rosabrook	• Obvious leaf mark • Slightly hairy runners • Stipules are green
Woogenellup	• Hairless runners • Hairy petioles (potentially) and peduncles • Stipule has red stripes • Light leaf markings • Large light green leaves • Long petioles

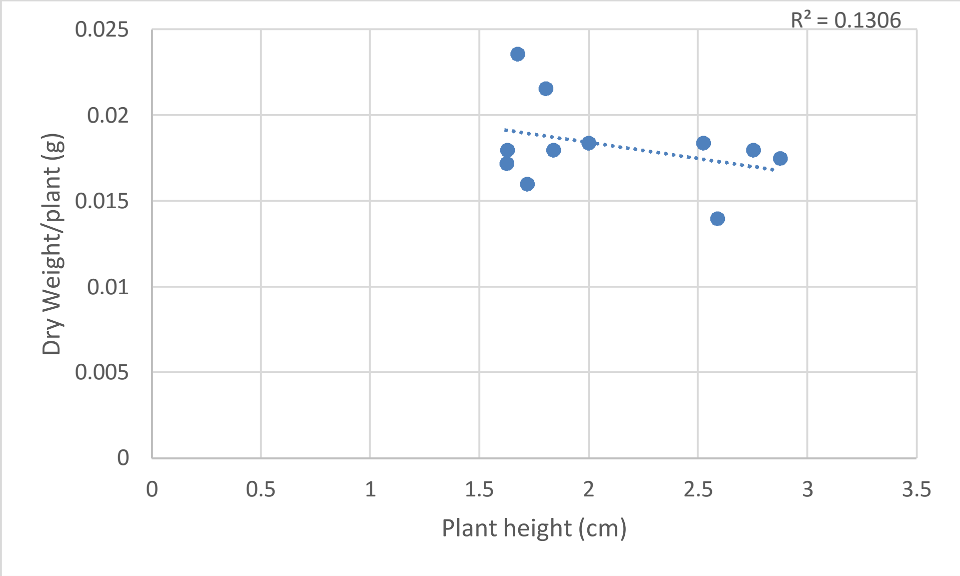

Figure A.2: Relationship between sub clovers height at harvest on May 5th and dry weight

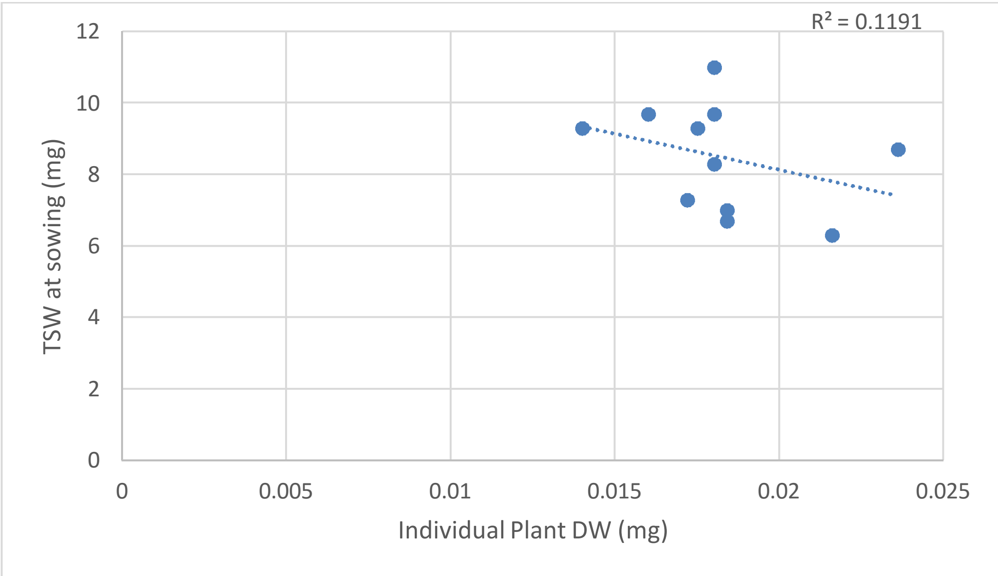

Figure A.3: Relationship between sub clover seed weight at sowing and individual dry weight

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