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Ecology and Management

of Adventive Annual clover species in the

South Island hill and high country of New Zealand

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
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Lincoln University
2013
Increasing legume abundance is an important component of pastoral intensification, in providing increased quality feed and nitrogen inputs to nitrogen deficient, hill and high country grassland. Establishment and persistence of traditionally sown clover species white clover (Trifolium repens L.) and subterranean clover (Trifolium subterraneum L.) is often limited in summer dry areas. The ecology of naturalised adventive (volunteer) annual clovers species Trifolium arvense L., Trifolium dubium Sibth, Trifolium glomeratum L., and Trifolium striatum L. commonly found in the South Island hill and high country areas of New Zealand was examined through a series of site surveys, field trials, and glasshouse experiments. The relationship of these clover species to environmental factors of climate (annual rainfall), topography (slope, altitude, aspect), soil fertility (phosphorus, sulphur, pH), and sheep grazing (spring management, and preference) were investigated.

On two contrasting hill and high country stations in the South Island: Glenfalloch in inland Canterbury (1665 mm annual rainfall) and Mt Grand in Central Otago (703 mm annual rainfall), the abundance of these species was quantified in relation to environmental factors. Site surveys were conducted in three hill blocks per farm, with measurements of grassland species cover, slope, aspect, grazing intensity, soil fertility, soil depth, and altitude, made within quadrats along three transects at upper, middle and lower hill slope positions. The single adventive clover present at Glenfalloch was Trifolium dubium, whereas all adventive species were present at Mt Grand. The % cover of adventive annual clovers was greater (30.1)
than that of white clover *Trifolium repens* (3.8) or subterranean clover *Trifolium subterraneum* (0.1) at Mt Grand. *Trifolium repens* % cover decreased with increasing altitude. Striated clover *Trifolium striatum* and cluster clover *Trifolium glomeratum* % cover was greatest on sunny aspects, while *Trifolium repens* % cover was greatest on shady aspects. *Trifolium repens* cover increased and *Trifolium striatum* cover decreased with increasing available soil phosphorus (P). Annual rainfall appeared to be the main environmental factor influencing the abundance of adventive annual clover species on two geographically and climatically different South Island high country stations.

A two year spring grazing management and superphosphate (SP) application field trial was conducted at a typical summer dry pasture site on a mid altitude (~700 m a.s.l.) north-facing moderately steep, hill site at Mt Grand Station. Adventive annual clover biomass, botanical composition and population dynamics were measured from October 2008–May 2010, from low (75 kg/ha) and high (200 kg/ha) SP application split plots of 2.5 x 5 m area within different grazing management main plots (continuous and mid-spring closure) of 5 x 5 m area. Adventive annual clover species contributed the largest proportion of legume sward content at this site, with sown species contributing the least. Collectively, adventive annual clover species accounted for over 90% of the sward clover content present during both years. *Trifolium striatum* was the dominant adventive species. Large variation in biomass production (668 and 51 kg DM/ha), sward botanical contribution (28 and 2%) and seedling recruitment (9.7 and 24 seedlings/core) by adventive annual clovers was observed between the two years of sampling, which was attributed to high rainfall during spring and early summer of the first year compared to the very dry spring of the second year. Deferred spring grazing resulted in significantly more adventive annual clover biomass in a moist year (1452 kg DM/ha) in comparison to continuous grazing (674–805 kg DM/ha). However, this did not appear to result in significantly more adventive annual clover seed production, seedling recruitment, or biomass in the following year. Superphosphate (SP) application had no significant overall influence on adventive annual clover species biomass. High SP application did have a negative effect on autumn seedling recruitment of the three most prevalent adventive annual clover species (*Trifolium striatum*, *T. dubium*, and *T. glomeratum*).

Soil plant-available N sourced from pasture legumes has been shown to be positively associated with long-term use of fertiliser phosphorus (P) and sulphur (S) use in NZ high and hill country. Determining the nutrient requirements of little known naturalised, adventive pasture legume species such as cluster clover (*Trifolium glomeratum*), haresfoot clover (*T.
arvense), striated clover (T. striatum) and suckling clover (T. dubium) that exist on low fertility, summer dry hill and high country slopes is important for the productive pastoral sustainability of extensive livestock grazing agroecosystems. The first glasshouse study was conducted to determine the yield response of these four adventive annual species, plus *Trifolium subterraneum* and *T. repens*, to increasing levels of available P in a typical, low fertility, acidic NZ high country soil. The order of greatest yield DM response was *T. subterraneum* > *T. arvense* > *T. repens* > *T. dubium* > *T. glomeratum* > *T. striatum* (4.4–0.8 g DM/pot), while the P application rates at which maximum yield was produced varied between the species with *T. arvense* and *T. striatum* yielding the most DM at 250 mg P/kg soil, *T. subterraneum*, *T. glomeratum* and *T. repens* yielded the greatest amount at 500 mg P/kg soil, and *T. dubium* producing its highest yield at 2500 mg P/kg soil. The order of greatest P-response efficiency by species (how much P was required for each gram of DM produced) was *T. subterraneum* > *T. arvense* > *T. repens* > *T. glomeratum* > *T. dubium* > *T. striatum*. Implications for low input, extensive grazing systems in hill and high country areas are discussed.

The growth response and nutrient uptake of four adventive annual clovers to applied S or lime (CaCO₃), grown in a typical low fertility South Island (S.I.) high country soil, were investigated under glasshouse conditions and compared to white and subterranean clovers as reference species. The annual species had yield responses of 12–17%, or were unresponsive, to S applications. Maximum yields were generally in the order of *T. subterraneum* ≥ *T. arvense* ≥ *T. striatum* ≥ *T. dubium* ≥ *T. glomeratum* > *T. repens* (8.0–5.2 g DM/pot). For lime treatments, yields were strongly driven by P availability, linked to soil pH. *Trifolium repens* and *T. striatum* responded to liming at low lime rates (1 t/ha equivalent), while all other species had negative yield responses to liming. The data indicate that the adventive annual clovers are better adapted to low soil fertility (low pH and S) conditions, which in turn may be an important factor contributing to their success under S.I. high country field conditions.

Grazing preference of Merino sheep for the adventive annual clover species was investigated in a field trial at a dryland pasture site on the Canterbury Plains at Lincoln University. The quantity of clover species sward biomass removed during grazing and the time spent grazing from different clover species swards was quantified during grazing preferences tests in November and December. Relative preference (Chesson-Manly index) for each clover species sward on offer was calculated from the utilisation of each species relative to the utilisation of all other species on offer. Relative preference decreased and was significantly different
between species when they reached a reproductive stage of maturity. This was attributed to an overall decrease in nutritive value of clover herbage, with relative preference significantly correlated with the concentration of acid detergent fibre (ADF), neutral detergent fibre (NDF), protein, and dry matter digestibility (DMD) chemical components. The latest flowering clover species were the most preferred, with *Trifolium repens* the most preferred clover species overall. *Trifolium arvense* was the most preferred adventive clover species when most species were predominately vegetative. Implications of key results and further research avenues are discussed.

Dedication

This PhD thesis is dedicated to my dear friends, Angela Hammond, and her late husband Matthew Lynn Hammond, respected Taranaki sheep and beef farmers and the once owners of Piri Piri Station, a North Island steep hill country Romney sheep and Angus cattle farm in Awakino. While working as a farmhand at Piri Piri Station on their invitation, their kindness towards me, and their wise, patient, and firm mentorship was a primary cause for my acquired-love of pastoral agriculture, and deep interest in grazed hill country environments.

To my late grandmother, Mary McNab Maxwell, for ever encouraging me and motivating me to do always do my best.

To my late friend, Jordan Alexander McKay.

To my late PhD colleagues, Jens Richardson and Maxwell Mathabiswana.
Acknowledgements

The Miss E L Hellaby Indigenous Grasslands Research Trust is acknowledged for generous funding, without which this PhD study would not have been possible.

I would like to acknowledge my excellent supervisors, Professor Grant R Edwards, and Dr Jim L Moir. I do not think that I could have asked for better qualified men to guide and advise me through my doctoral study in the realm of hill and high country pasture ecology.

Thanks must go to Mr Richard J Lucas for his infectious passion for pasture legumes in NZ dry hill and high country, and his enthusiastic, assertive and down-to-earth advice, and positive encouragement during my PhD study.

Thanks to Professor Derrick J Moot for valuable advice and encouragement, and for never being shy to give constructive criticism for the benefit of my doctoral study.

I would like to thank Dr Keith M Pollock for technical assistance, and for our always pleasant and thoughtful dialogues on Mt Grand Station hill and high country grassland ecology, which benefited my holistic understanding of the grassland agroecosystem I have spent four years studying.

Thanks to Dr Racheal H Bryant for advice and insight on my grazing preference study, statistical assistance and encouragement.

Thank you to Mr Colin Pettigrew, farm manager of Lincoln University’s Ashley Dene dryland sheep farm, for providing Merino sheep for my grazing preference trials.

Many thanks go to members of the Lincoln University Field Service Centre staff for their technical assistance and cheerful professionalism during my study; Mr Dave Jack, Mr Daniel Dash, Mr Don Heffer, Mr Allan Marshall, Mr Malcolm Smith, Mrs Vonny Fasi, Mrs Kim Barnes, and Ms Anna Mills. Thank you to Mr Brent Richards and Mrs Leona Meachen of the Lincoln University Plant Nursery Centre for looking after my clover pots. Thank you to Carole Barlow, Angela Reid, and Lynne Clucas of the Department of Soil and Physical Sciences at Lincoln University for technical assistance.

Thank you to Chas and Dietlind Todhunter of Glenfalloch Station for provision of high country soil for both my glasshouse soil fertility experiments.

I would like to thank Mr Evan Gibson, the former farm manager of Lincoln University’s Mt Grand Station, for his valuable assistance and essential contact during visits to my hill side grazing experiment site visits at Mt Grand for data collection.

Gratitude goes to friends and fellow students who assisted me during my experiment maintenance, data collection, processing and analysis; Dr Yoshitaka Uchida, Fiona McConville, Jacob Henson, Sho Kasuya, William Schultz, Sarena Che Omar, Kalif Ismail, Elina Stang, Gerrard Pile, Graham Swanepoel, Steele Taylor, Kate Chapman, Tafadzwa Marerwa, and Francis Malcolm.
Many thanks to my Lincoln University postgraduate student mates and colleagues Dr Michael Cripps, Dr Shokri Jusoh, Frisco Nobilly, Walthor Dumin, Dr Innocent Rugoho, Omar Al-Marashdeh, Dr Paul Long Cheng, Daniel Colluci, Yang Guo, Dr Jin Han, and Dr Mimi Sueng Ok Byun, Dr Damian Bienkowski, Saman Berenji, Dr Arash Rafat, Amir Daryaei, Hoda Ghazali, Juan Dvenas-Serrano, Alana Harrison, and Fernando Garcia Barrios for support and encouragement.

Many thanks go to my good friends Sho and Anna Kasuya, Roger Labrum, Christopher Ying, Thomas Reed, Ashley Jannesen, Curtis Lambert, Kate Schowe, Joe Good, Elizabeth Barkla, Marie Neumann Andersen, Ask Møller, Jesper Lehmann, Jenny Johnsen, Elina Stang, Hilde Lysaker Næss, Sarena Che Omar, and Akshay Pahuja, here in New Zealand, Hong Kong, and overseas, for their encouragement and support.

I would like to say thank you to my friends from the Young Men’s Division of the Soka Gakkai International NZ; Shin Kaleohano Louis, Yu Ito, Tomo Shibata, and Susumu Irie, and from the Men’s Division of SGI-NZ; Steven Yee, Masa Takemura, and Nao Ota, for their support and encouragement during my PhD study.

I would like to express gratitude to Kimberley Jane Pulley for her encouragement and support during my PhD study, and pushing me to complete my thesis writing.

To all my family in Hong Kong for their love, unwavering support and encouragement; special mention goes to my cousins Bonnie and Winnie, and her husband Mr Kevin Wong.

Much appreciation and gratitude go to all my family in New Zealand: my sister Alison, brother-in-law John Davies, my nephews Montrose and Nico, my niece Anysia, and to my aunt and uncle Mary and Gary Taylor, for their love and unwavering support and encouragement throughout my doctoral study. Your support has been invaluable. I express gratitude to Jenny Strother and Ross Parsonage for showing me support, and statistical help with data analysis.

Great appreciation and gratitude go to my loving Mum and Dad, Mrs Greta Chor Wan Maxwell and Professor Gordon Selwyn Maxwell, for their love, encouragement, and unwavering support of every form during my doctoral study.
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Chapter 1
General Introduction

1.1 Background

Economic return from commodities produced from high country grazing systems is under pressure following retirement of summer-grazing high altitude areas (>1000 m altitude). The aim of this land retirement is to improve indigenous grassland conservation (preservation) and recreation resources, driven by central government policy (O’Connor 2003; Norton 2004; Barratt et al. 2006; Gillespie 2006; Brower 2008). The general trend of decreased wool prices in recent decades (Meat and Wool New Zealand Economic Service 2009) has resulted in a change from wool production as the main source of farm income, to store lamb and calf production in conjunction with wool as a means to increase hill and high country farm income (FAO 2009). Furthermore, recent land use conversion in lowland areas from intensive sheep and beef finishing (fattening) to dairying and dairy grazing has forced hill and lower altitude high country areas to intensify (FAO 2009) as fattening destinations for store lamb and cattle from hill and high country farms are being displaced by these dairy conversions. The economic and agro-ecological sustainability of high country pastoralism depends on improvement of both the productive capacity of remaining middle and lower altitude grassland (900–300 m altitude) of various hill slope and aspects, and the current pasture management of such areas used for extensive livestock grazing in predominately merino (fine) wool, lamb and beef production enterprises is therefore required (O’Connor 2003; Maxwell et al. 2010).

Grazed grassland productivity in New Zealand hill and high (>700 m altitude) country is driven strongly by nitrogen (N) inputs sourced primarily from sward legumes and the associated biological N fixation (Haynes & Williams 1993). The principal nutrient driving pasture growth in pasture systems is nitrogen (Chapman & Macfarlane 1985). Most hill and high country soils are low in available N (White 1990) thus limiting pasture production. Nitrogen fertiliser trials strongly demonstrate that livestock feed production in hill country and lower altitude high country is dependent on N inputs (Gillingham et al. 2004; Smith et al. 2004). The traditional model of pasture improvement in New Zealand has been the introduction of legume species and/or enhancement of the overall legume abundance with the purpose of increasing both N
inputs to N-deficient grassland and the nutritional value of available herbage for livestock (Bowatte et al. 2006). Therefore, increasing and then maintaining the legume component within the grazed sward is an inherent objective of pastoral improvement (intensification) for hill and high country grassland communities and important for sustainability of grazed high country (Boswell et al. 2007).

Improved legume-based pastures are especially important within summer dry (<800 mm rainfall per annum) eastern regions of New Zealand, where seasonal soil moisture deficits caused by evapotranspiration exceeding summer rainfall (Brown & Green 2003) constrains potential pasture production. In such dryland environments maximizing pasture productivity and quality during the period of reliable soil moisture (autumn to mid-spring) is essential (Gillingham et al. 2003). Establishment and persistence of broadcast-sown legume species (white clover *Trifolium repens* L. and subterranean clover *Trifolium subterraneum* L.) is often limited (Knowles et al. 2003; Power et al. 2006) in hill and high country grassland agroecosystems of dry eastern areas within the North Island’s East Coast, Gisborne, Hawke’s Bay, Wairarapa, and the South Island’s Marlborough, Canterbury and East Otago regions, including inland areas of South Canterbury and Central Otago, despite repeated oversowing particularly with *T. repens* (Power et al. 2006).

Contrastingly, other naturalized but unsown and adventitious (volunteer) annual legumes; haresfoot clover *Trifolium arvense* L., suckling clover *Trifolium dubium* Sibth, cluster clover *Trifolium glomeratum* L. and striated clover *Trifolium striatum* L. are widely distributed within the South Island high country (Hoglund 1990; McIntosh & Allen 1998; Gravuer 2004; Rose & Frampton 2007) and are commonly present to locally abundant in summer dry zones, specifically on north-facing slopes of hill and high country areas (Boswell et al. 2003a; Power et al. 2006; Maxwell et al. 2010). Most adventive annual clover species are considered small in stature, and associated generally with lower soil fertility conditions and hence lower pasture productivity (Scott 2003). Information as such regarding biomass productivity of these four adventive clover species is limited in New Zealand however, partly because they are expected to grow in conditions considered suboptimal for *T. repens* production (Boswell et al. 2003a). Little research has been conducted looking at the growth, dry matter (DM) production, and nutrient (phosphorus and sulphur) and lime response of adventive annual clover species either in climate-controlled studies or field trials. Detail in the literature on the grazing management response, annual DM
production, and regeneration from seed in a high country environment, and grazing preference of these species by sheep appears limiting or is scarce.

The primary aim of this thesis is to attempt to address livestock feed production issues associated with decreasing high country pastoral land area and associated land areas (lamb and cattle finishing) as a result of high country land tenure review (O’Connor 2003) and dairy industry expansion (FAO 2009; Maxwell et al. 2010). Critically important in achieving successful pasture intensification is the effective choice of legume species, good grazing, and soil fertility management to promote their growth. The primary objective of this thesis therefore is to determine some of the factors controlling the abundance, growth, and population dynamics of four annual clover species (haresfoot clover *Trifolium arvense*, suckling clover *Trifolium dubium*, cluster clover *Trifolium glomeratum* and striated clover *Trifolium striatum*) that are naturalised in New Zealand’s South Island hill and high country. Sown species white clover *Trifolium repens* and subterranean clover *Trifolium subterraneum*, are used a reference species to compare with the naturalised annual species, throughout the thesis.
1.2 Objectives and thesis structure:

1. Quantify the abundance of six clover species (haresfoot clover, suckling clover, cluster clover, striated clover, subterranean clover, and white clover) in relation to climate, topography, soil fertility and management on extensive hill and high country grazing blocks on two South Island hill and high country farms.

2. Determine, in a field trial, the effect of grazing management and sulphur & phosphorus soil fertility on the botanical composition, dry matter production, and reproductive performance of six clover species (haresfoot clover, suckling clover, cluster clover, striated clover, subterranean clover, and white clover).

3. Quantify the dry matter response of six clover species (haresfoot clover, suckling clover, cluster clover, striated clover, subterranean clover, and white clover) to phosphorus fertilizer application in a low fertility high country soil, grown under glasshouse conditions.

4. Quantify the dry matter response of six clover species (haresfoot clover, suckling clover, cluster clover, striated clover, subterranean clover, and white clover) to sulphur fertilizer and lime application in a low fertility high country soil, grown under glasshouse conditions.

5. Determine the grazing preference of Merino sheep for six clover species (haresfoot clover, suckling clover, cluster clover, striated clover, subterranean clover, and white clover) at two different stages of maturity.

This thesis comprises eight Chapters. Following this general introduction chapter, a comprehensive literature review is presented in Chapter 2. Chapter 3 is a conference paper published in the Proceedings of the New Zealand Grassland Association (2010) and reports the findings of two high country station site surveys where the abundance of clover species present in transect quadrats was related to climatic (annual rainfall), edaphic (soil fertility and depth), and topographic (slope angle, aspect, and altitude) factors. In Chapter 4, the results of a two-year field
trial investigating the influence of different spring grazing management (continuous and deferment; closure) and superphosphate (P and S) fertiliser application on adventive annual clover botanical composition, DM production, and population dynamics (seed production and seedling recruitment) at a typical summer dry (<800 mm rainfall per annum) high country site are presented. The results and interpretations of two glasshouse studies determining the P, and S and lime requirements of the four adventive annual clover species and Trifolium repens and T. subterraneeum are presented in Chapter 5 and 6 respectively, with discussion on nutrient uptake and nutrient-use efficiency in relation to low fertility pasture environments. Chapter 6 is a journal paper published in the New Zealand Journal of Agricultural Research, Volume 55:1 (2012). The results and interpretations of a field experiment investigating the grazing preference of Merino sheep for six clover species (four adventives and two commonly sown species, T. repens and T. subterraneeum) are presented in Chapter 7. Here, a relative preference value for each clover species indicating whether a species was more preferred or least preferred, at two different stages of maturity, are related to parameters of chemical composition of clover herbage, with implications for the presence of adventive annual clover species in grazed hill and high country discussed. Finally, the key results of this thesis and the role of adventive annual clover species are discussed in Chapter 8, with implications of the main research findings and avenues for further research presented.
Chapter 2
Literature Review

2.1 Annual clover species

2.1.1 ‘Adventive’ and ‘Naturalised’ terms

This thesis uses two ecological terms ‘adventive’ and ‘naturalised’ in reference to the four annual clover species *Trifolium arvense* L., *T. dubium* Sibth, *T. glomeratum* L., and *T. striatum* L. that are present in New Zealand, and the focus of this doctoral study. More often than not, this thesis uses ‘adventive’ in singular and/or collective reference to these four annual clover species. The primary reason for this choice is that within New Zealand, and amongst the New Zealand literature, the word ‘adventive’ has been widely used to describe these clover species (Boswell et al. 2003a; Power et al. 2006; Lonati et al. 2009).

The word ‘adventive’ derives from the Latin ‘*adventus*’, meaning arrival / advent, and describes an organism transported into a new habitat, whether by natural means or the agency of man (Lincoln, Boxhall & Clark 1998). ‘Adventive’ flora is distinct from native and cultivated floras, and consists of plant species that grow spontaneously outside cultivation (Te Ara Encyclopaedia of New Zealand 1966). ‘Adventive’ plant species are significant in their role in forming large vegetation communities, either distinctly, or in combination with native species (Te Ara Encyclopaedia of New Zealand 1966).

A naturalised organism is an alien or introduced species that has become successfully established (Lincoln, Boxhall & Clark 1998). Naturalised species reproduce consistently and sustain a population over many life cycles without direct intervention by humans (or in spite of human intervention); they often recruit offspring freely, usually close to adult plants, and do not necessarily invade natural, semi-natural or human-made ecosystems (Richardson et al. 2000).

The author acknowledges that some definitions of ‘adventive’ plant species, outside New Zealand contexts and literature, further describe ‘adventive’ species as introduced organisms that are not fully established in a new habitat or environment, are not self-sustaining, and whose numbers are only increased by non-reproductive means (Wikipedia). This is in comparison to a ‘naturalised’
species, that has been introduced to a region and their reproduction is sufficient to maintain a population. The annual clover species studied in this thesis appear to fit the definition of ‘naturalised’ plant species more closely than that of an ‘adventive’ species, with regards to the latter not being self-sustaining and regenerating or spreading by non-reproductive means. The reviewed literature and results of this thesis however, state and show otherwise.

Invasive plant species are categorised based on the ability to sustain self-replacing populations over several life cycles; produce reproductive offspring; and have the potential to spread over long distances (Richardson and Pysek 2012). Invasive species must therefore be introduced, survive, reproduce, disperse and spread (Richardson et al. 2000; 2011; Blackburn et al. 2011). Plant species that only form self-sustaining populations and do not spread substantially are naturalized but not invasive. It could be strongly argued, based on the reviewed literature, results, and discussions within this doctoral study, that the above description of an invasive species accurately describes these four annual clover species in New Zealand today.

However, the purpose of this thesis was not to redefine or clarify the nomenclature of these non-indigenous annual clover species, or more fundamentally, scrutinise the ecological terms surrounding exotic, non-indigenous, and invasive species, around which ambiguity exists (Colautti and MacIsaac 2004). The most detailed literature available on these four clover species in New Zealand, prior to completion of this thesis, was by Boswell et al. (2003a) who addressed these species throughout their review as annual clovers ‘adventive’ in New Zealand summer dry hill and high country.

2.1.2 General description

Approximately 20 annual clover species have established within New Zealand (Scott 2003) of which subterranean clover (*Trifolium subterraneum* L.) is considered the most important annual clover species in dryland environments. However, it is generally regarded as a sown species in developed pastures rather than an adventive annual clover species (Boswell et al. 2003a). Annual clovers under the naturalised category of legume species related to New Zealand dryland agriculture are species already present in NZ, as a result of accidental arrival or deliberate introduction for various purposes, but have since escaped cultivation and are successfully maintaining small populations (Webb et al. 1988). Many annual legumes are present within the
adventive flora and come from plant introduction programs, with several species being widespread, for example haresfoot clover (*Trifolium arvense* L.) in dry environments, and suckling clover (*Trifolium dubium* L.) in moister areas (Scott 2003).

Boswell et al. (2003a) stated that the most common non-commercial annual clovers, under the naturalised adventive species category, are suckling clover (*Trifolium dubium* Sibth), cluster clover (*T. glomeratum* L.), striated clover (*T. striatum* L.), and haresfoot clover (*T. arvense* L.). The main attribute of adventive annual clovers within New Zealand’s pastoral agriculture is to fix atmospheric nitrogen into plant nitrogen, thus improving overall pasture quality and increasing the nitrogen available to other plants in the pasture community. Scott (2003) commented that most naturalised annual clovers are small in stature, and are generally associated with lower fertility conditions and the lower productivity associated with such conditions. In addition, these clovers fluctuate widely in prominence with climate variation between years (K. Pollock pers. comm.).

### 2.1.3 Origin and geographic range

*Trifolium arvense* L. (haresfoot trefoil or haresfoot clover) occurs naturally from Europe to Asia Minor, Caucasus, North and West Asia, and North Africa (Webb et al. 1988). Within New Zealand the species is locally common in the North Island, especially in Hawke’s Bay and dry areas of Wellington. In the South Island, it is common to abundant in dry areas of Marlborough, Canterbury and Otago, such as waste places, pasture, grassland, riverbeds, coastal situations, and cultivated land.

*Trifolium dubium* Sibth (suckling clover) has a native range that covers Europe to Caucasus. It is common throughout New Zealand, and can be found growing in a wide range of habitats (waste places, pasture, lawns, gardens, and cultivated land), (Webb et al. 1988).

*Trifolium glomeratum* L. (cluster clover) originates from southern and western parts of Europe, Asia Minor, Caucasus, and North Africa. It is locally common in dry pasture and waste places, cultivated land and coastal areas within the North Island (except in Taranaki) and the South Island, within Nelson, Marlborough, Canterbury, and Otago (Webb et al. 1988).
*Trifolium striatum* L. (striated clover) originates from Europe, North Africa, and the Mediterranean through to Caucasia and Iran. It is commonly found in dry waste places, grassland and pasture, coastal areas and cultivated land common in Wellington province and lowland Marlborough and Canterbury, and North and Central Otago districts (Webb et al. 1988).

### 2.1.4 Ecological strategies of annual clovers

Annual clovers are considered to be most competitive in New Zealand’s semi-arid environments, which are located in the rain shadow of the alpine ranges, inland, >350 metres above sea level (m a.s.l.), and experience warm summers and cold winters. They can also be found in dry environments on the northern faces of hill country in both islands and on eastern lowland shallow, stony soils from Hawke’s Bay to North Otago (Boswell et al. 2003a).

Carter (1984) listed four characteristics of most successful annual plants:

1. Germinate rapidly
2. Emerge rapidly
3. Prostrate growth habit to avoid ‘full’ effects of grazing
4. Set large numbers of small seeds under normal grazing and seasonal conditions.

Rapid germination and emergence of an annual plant seedling is advantageous towards utilising sunlight, moisture and nutrients for rapid growth during spring and reaching maturity quickly. Avoidance of ‘full’ grazing effects by having a prostrate growth habit does not mean avoidance of ‘all’ grazing effects, but the avoidance of the most or greatest influence of grazing. A prostrate growth habit can perhaps limit the detrimental effects of grazing. Carter et al. (1982) stated seed production and hard-seededness are crucial in annual legume persistence. In the same vein of describing the nature of clovers, Sheath & Macfarlane (1990) stated that the concept of persistence in relation to annual legume species is appropriately considered in terms of population regeneration. Therefore, reseeding, germination and seedling survival are important factors in the persistence of annual clovers. In terms of seed production, all four of the adventive annual clovers of interest are aerial seeders and produce generally small seeds (Table 2.1) of numerous quantities. Striated clover *Trifolium striatum* is the one exception to the small seed rule. These adventive species shed their seed on the soil surface in contrast to subterranean clover, of which a high proportion of seed is buried in the soil where seedling establishment will
generally be more assured. Adventive annuals rely on high numbers of small and hard seed for survival (Boswell et al. 2003a).

Table 2.1 Seed sizes of four adventive annual clover species in New Zealand, with subterranean (*Trifolium subterraneum*) and white (*Trifolium repens*) clovers as reference species (from Boswell et al. 2003a).

<table>
<thead>
<tr>
<th>Species of <em>Trifolium</em></th>
<th>Seed weight (mg)</th>
<th>Seed diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. arvense</em> (haresfoot)</td>
<td>0.23–0.44</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>T. dubium</em> (suckling)</td>
<td>0.42</td>
<td>1–1.5</td>
</tr>
<tr>
<td><em>T. glomeratum</em> (cluster)</td>
<td>0.33</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><em>T. striatum</em> (striated)</td>
<td>2.0</td>
<td>1.5–2.0</td>
</tr>
<tr>
<td><em>T. subterraneum</em> (subterranean)</td>
<td>4.3–12.40*</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td><em>T. repens</em> (white)</td>
<td>0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Dependant on cultivar

Successful germination and seedling establishment are dependent on adequate soil moisture at an appropriate time. For example, Boswell et al. (2003b) reported a huge variability between years in the germination, productivity and N fixation of haresfoot clover *Trifolium arvense* in semi-arid tussock grassland sites near Benmore and Omarama in the Mackenzie Basin region. In extremely dry years, *T. arvense* may not be evident on the landscape due to minimal germination of seed, while in moist seasons whole hillides may have a pinkish shade or appearance with the flowering of *T. arvense* during summer. Subterranean clover *Trifolium subterraneum* relies on the production of hard seed and high temperature dormancy to avoid false strike after summer rain and possible subsequent autumn drought. Like *T. subterraneum*, striated clover *T. striatum* in North Canterbury (Smetham 1980) and cluster clover *T. glomeratum* in lowland stony soils (R. Lucas pers. comm.) are winter active; they germinate in autumn, and can grow through winter and rapidly in spring. Other annuals, especially *T. arvense*, can germinate at any time when soil moisture and temperature conditions are favourable. Along with *T. arvense* and the other small-seeded species (*T. glomeratum* and suckling clover *T. dubium*), germination in spring is more reliable than germination in autumn in dry (semi-arid) higher altitude New Zealand environments (Boswell et al. 2003a).
2.2 Plant characteristics

A summary of the plant characteristics of the four adventive annual clover species and their habitat ranges within New Zealand is given in Table 2.2.

Table 2.2 Summary details of leaf and flower structures, plant form and geographical range of four adventive annual clover species in New Zealand (from Boswell et al. 2003a).

<table>
<thead>
<tr>
<th>Plant characteristics</th>
<th>T. arvense</th>
<th>T. dubium</th>
<th>T. glomeratum</th>
<th>T. striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Soft, greyish hairy leaflets</td>
<td>Hairless to slightly hairy; leaflets often reddish</td>
<td>Hairless</td>
<td>Dentally hairy; light green colour</td>
</tr>
<tr>
<td>Narrow leaflets</td>
<td></td>
<td>Stalk of central leaflet longer than others; some leaflets notched</td>
<td>Leaflets small, 3–12 mm long; some have single light or dark spot on leaves</td>
<td>Leaflets short-stalked, at times diamond-shaped, elongated 5–20 mm</td>
</tr>
<tr>
<td>Stems</td>
<td>Often reddish, wavy stems</td>
<td>Stems many, thin, wavy</td>
<td>Wiry, prostrate, scrambling</td>
<td>Stems hairy</td>
</tr>
<tr>
<td>Flower heads</td>
<td>Soft, hairy, pinnate, cylindrical; mainly at end of stem; many</td>
<td>5–12 flowers/head; flowers in leaf axil</td>
<td>Small globe-shaped heads; not stalked; in leaf axils</td>
<td>Egg-shaped heads 1.8 cm long; not stalked; at end of branches and in leaf axils</td>
</tr>
<tr>
<td>Flowers</td>
<td>Flowers pink-white, in distance flowers look like pink haze. Flowers Aug–May depending on habitat and season</td>
<td>Yellow flowers; flowers Oct–June depending on habitat and season; peak flowering Nov in most environments</td>
<td>Pink/purplish flowers, small flowers Nov–March depending on habitat and season</td>
<td>Flowers longer than calyx. Calyx ribbed, toothed, papyry</td>
</tr>
<tr>
<td>Plant form</td>
<td>Varies from single stem (10 cm tall; single flower head) to a plant with many stems and heads, 30cm across, 30 cm tall; form depends on habitat conditions</td>
<td>Few stems to many, circular prostrate form to semi-erect to scrambling form under favourable conditions; form depends on habitat conditions</td>
<td>Varies considerably in size with habitat condition</td>
<td>Prostrate in dry places; more erect in moist places</td>
</tr>
<tr>
<td>Plant characteristics</td>
<td>Germinates when moisture temperatures are satisfactory, mainly spring</td>
<td>Winteractive North Island; autumn germination</td>
<td>Germinates when moisture; temperatures are satisfactory; autumn germination in Canterbury</td>
<td>Winteractive N. Canterbury; autumn germination in dry Places is short-lived; leaves wither and the prickly budling calyx at nodes give feeling of corms with knots at intervals (hence knotted cover)</td>
</tr>
<tr>
<td>Range</td>
<td>Common to locally abundant in dry waste places, river beds, modified tussock grassland at low altitudes, pastures on light soils, both islands</td>
<td>Common throughout New Zealand</td>
<td>Locally common at dry waste places and pasture and coastal areas in eastern areas of both islands to North Otago</td>
<td>Occasional to locally abundant in dry waste places and thin hill pastures, or light soils in both islands, especially drier east-coast areas, and modified tussock grassland of east St.</td>
</tr>
</tbody>
</table>

2.2.1 Germination

Boswell et al. (2003a) stated that the timing and rate of germination are crucial factors in the re-establishment of competitive winter annual seedlings. Year to year variability in the arrival of autumn rainfall means the ambient temperatures experienced by seed will differ annually. Ultimately, adventive annual clover germination is dependent on a combination of adequate soil moisture and favourable temperatures. No evidence thus far, suggests that a pre-chilling treatment for germination is required for the four adventive annuals (Boswell et al. 2003a).
Lonati et al. (2009) used thermal time ($T_t$, °C days) to quantify germination, seedling emergence (defined as the time of appearance of the spade leaf), and first trifoliate leaf appearance of the four adventive annual clover species, *T. subterraneum* and *T. repens*. Base temperatures for germination of all five annual clover species were less than the base temperature for germination of *T. repens* of 2.5°C (Tables 2.3). All five annual clover species (four adventives plus *T. subterraneum*) had lower thermal time requirements for emergence and seedling development than the perennial clover species *T. repens* (Table 2.4), whose thermal time requirement for first trifoliate leaf appearance was 310°C days (Moot et al. 2003). The maximum number of seeds germinating was greater than 95% at all temperatures (Figure 2.1), indicating that no pre-chilling or additional chemical treatments were required for maximum germination of these species.
Table 2.3 Estimates of the base temperature ($T_b$), optimum temperature ($T_{opt}$), maximum temperature ($T_{max}$) and thermal time requirement for germination of clover species at sub-optimal ($T_{tub}$) and supra-optimal ($T_{tus}$) temperatures (adapted from Lonati et al. 2009).

<table>
<thead>
<tr>
<th>Species</th>
<th>$T_b$ (°C)</th>
<th>$T_{opt}$ (°C)</th>
<th>$T_{max}$ (°C)</th>
<th>$T_{tub}$ (°C days) ($T_b$ to $T_{opt}$)</th>
<th>$T_{tus}$ (°C days) ($T_{opt}$ to $T_{max}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trifolium arvense</em></td>
<td>0.4</td>
<td>11.8</td>
<td>25.6</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td><em>T. dubium</em></td>
<td>0.3</td>
<td>13.8</td>
<td>24.4</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td><em>T. glomeratum</em></td>
<td>-1.1</td>
<td>12.9</td>
<td>27.0</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td><em>T. striatum</em></td>
<td>1.4</td>
<td>11.6</td>
<td>27.7</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td><em>T. subterraneum</em> ‘Mt Barker’</td>
<td>0.1</td>
<td>13.0</td>
<td>27.0</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td><em>T. repens</em> ‘Demand’</td>
<td>2.5</td>
<td>c. 25</td>
<td>-</td>
<td>37</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.4 Thermal time requirements for emergence ($T_{tep}$), first trifoliate leaf appearance ($T_{tr}$), initiation of stolons ($T_{br}$) and phyllochron of the five annual clovers in a controlled environment experiment (mean ± standard error). Within column values followed by different letters are significantly different. *$P \leq 0.05$; ***, $P \leq 0.001$; NS, not significant (from Lonati et al. 2009).

<table>
<thead>
<tr>
<th>Species</th>
<th>$T_{tep}$ (°C days)</th>
<th>$T_{tr}$ (°C days)</th>
<th>$T_{br}$ (°C days)</th>
<th>Phyllochron (°C days/leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trifolium glomeratum</em></td>
<td>165 ± 4.7 a</td>
<td>243 ± 8.1 a</td>
<td>447 ± 4.9 a</td>
<td>60 ± 5.2</td>
</tr>
<tr>
<td><em>T. striatum</em></td>
<td>160 ± 8.2 ab</td>
<td>234 ± 4.6 ab</td>
<td>355 ± 0.0 b</td>
<td>52 ± 0.8</td>
</tr>
<tr>
<td><em>T. subterraneum</em> ‘Mt Barker’</td>
<td>160 ± 0.0</td>
<td>220 ± 4.6 b</td>
<td>437 ± 8.2 a</td>
<td>55 ± 2.5</td>
</tr>
<tr>
<td><em>T. dubium</em></td>
<td>146 ± 0.0 ab</td>
<td>220 ± 4.6 b</td>
<td>341 ± 8.4 a</td>
<td>62 ± 3.8</td>
</tr>
<tr>
<td><em>T. arvense</em></td>
<td>141 ± 4.7 b</td>
<td>220 ± 4.6 b</td>
<td>351 ± 4.7 b</td>
<td>53 ± 1.5</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>NS</td>
</tr>
</tbody>
</table>

The responses in Figure 2.1 allowed calculation of the cardinal temperatures for these adventive species using the methodology of Moot et al. (2000) in order to show germination response at any temperature, or range of temperatures experienced in the field (Figure 2.2). These results strongly reflect the adaptation of adventive annual clover species to cool moist autumn conditions during germination. Maximum temperature for germination ($T_{max}$) and optimum temperature for germination ($T_{opt}$) values can be utilised for any site and season to give an indication of when these species can be expected to germinate after autumn rain.
Figure 2.1 Cumulative germination (%) of A, *Trifolium arvense*; B, *T. dubium*; C, *T. glomeratum*; D, *T. striatum*; E, *T. subterraneum* ‘Mt Barker’; F, *T. repens* ‘Demand’ seeds at 4.6 (●), 9.2 (○), 9.6 (▼) 14.8 (▲), 19.5 (■), and 25.0 (□°C. Bar = maximum standard error for the final germination percentage (from Lonati et al. 2009).
Lonati et al. (2009) stated that after autumn rainfall in a Mediterranean climate these species can be expected to germinate, rapidly develop a leaf canopy, and have the ability to reach a sufficiently advanced stage of seedling development before the onset of low winter temperatures. For example, *T. striatum* germination would be expected within 2 days at a mean soil temperature of 14°C, but after 7 days at 4°C or 5.5 days at 20°C (Figure 2.2). The low optimum and $T_{\text{max}}$ temperatures highlight the adaptation of *T. striatum* to cool moist conditions and high temperature dormancy, in contrast to *T. repens*. Further, at a mean daily temperature of 7°C, *T. arvense* is predicted to germinate in about 3 days ($T_{\text{sub}} = 23°C$ days); emerge (spade leaf visible) after 20 days ($T_{\text{sp}} = 141°C$ days; Table 2.4), and produce the first trifoliate leaf after 31 days ($T_{\text{tr}}$...
The consistent phyllochron of ~53°C days (Table 2.4) means the second trifoliate leaf should appear after about another 8 days. *Trifolium arvense* can thus be expected to have a seedling with four leaves (spade leaf + three trifoliate leaves) and be commencing secondary and stolon leaf initiation in ~50 days (350°C days; Figure 2.3). In comparison, *T. repens* has greater accumulated *Tt* requirements for germination (37°C days; Table 2.3) and emergence of the first (*Tt* = 309°C days) and subsequent trifoliate leaves with a phyllochron of 107°C days per mean stem leaf which means its relative canopy expansion is delayed compared with the annual clovers (Moot et al. 2003).

![Figure 2.3](image)

**Figure 2.3** Numbers of trifoliate main stem leaves (●) and total trifoliate leaves (○) of five annual clover species: A, *T. arvense*; B, *T. dubium*; C, *T. glomeratum*; D, *T. striatum*; E, *T. subterraneum* ‘Mt Barker’. Arrows indicate the initiation of stolon development; bar = standard error for the final total leaf number (adapted from Lonati et al. 2009).
2.2.2 Tolerance of cold and moisture stress

Ehram & Cocks (1990) working in Syria showed that differences in environment governed the distributions of *Trifolium glomeratum*, *T. arvense* and *T. subterraneum*. Specifically, *T. arvense* is better adapted to lower rainfall (400–520 mm/year) and extreme temperature environments such as cold inland mountains and hot plains, when compared with *T. glomeratum* and *T. subterraneum*.

In an evaluation of the tolerance of 13 *Trifolium* species to single frost events of varying severity, conducted in a controlled environment room in which only the tops of clover plants were subjected to frosts going down as low as -16 °C, Caradus (1995) found that *T. arvense* and *T. dubium* were more frost tolerant, compared to *T. glomeratum* and *T. subterraneum* which were among the most sensitive or susceptible species to frost. *Trifolium striatum* and *T. repens* were intermediate in responses to frost treatment.

Power et al. (2006) compared the prevalence of annual clover species (*T. subterraneum*, *T. dubium*, *T. glomeratum*, *T. arvense* and *T. striatum*) and *T. repens* in relation to aspect, altitude, temperature and rainfall on Mt. Grant Station, Lake Hawea, during the late winter to early summer period (mid-August to December). The larger amount of *T. arvense* and no reduction in *T. dubium* observed at 910 m a.s.l on a northerly facing hill side by Power et al. (2006) was attributed to the frost tolerance of these species. Caradus (1995) reported that *T. arvense* and *T. dubium* were among the most frost tolerant clover species. Furthermore, the distribution of clovers at Mt. Grand sites (Figure 2.4) relates to some extent the seedling frost tolerances found by Caradus (1995). *Trifolium subterraneum* presence was minimal on the south facing site (630 m a.s.l) and perhaps reflected the more severe frost events experienced in contrast to warmer north facing sites (Power et al. 2006).
Figure 2.4 Percentage cover of cluster (*Trifolium glomeratum*), white (*T. repens*), sub (*T. subterraneum*), suckling (*T. dubium*), striated (*T. striatum*), and haresfoot (*T. arvense*) clovers at five sites at Mt. Grant Station, Central Otago, New Zealand during spring 2002 and December 2003. Sites were northerly-facing (N) at 450, 620, 750, and 910 m a.s.l. and south facing (S) at 630 m a.s.l. (from Power et al. 2006).

Beale et al. (1993) showed from a survey conducted in Morocco that clover species distributions were more influenced by rainfall rather than temperature. They found *T. arvense* present at low rainfall sites (575 mm), *T. striatum* at higher rainfall sites (773 mm), and *T. glomeratum* and *T. subterraneum* adapted to intermediate rainfall sites, at 685 and 670 mm respectively. Boswell et al. (2003a) stated that within New Zealand, annual clovers persist where there is regular moisture deficiency, such as on shallow, stony lowland soils, dry north-facing hills and the inland South Island semi-arid soils.
Power et al. (2006) reported that the percentage ground cover of annual clovers was dominant on the four sunny (N facing) sites at Mt. Grand. The authors stated that this was a demonstration of these adventive annual clovers’ greater adaptation to long dry summers in comparison to *T. repens*, and the benefits of reduced plant competition from the shorter and less dense grass sward. The dominance of perennial species (grass and clover) in the south facing pasture and annuals on the north faces was attributed by Power et al. (2006) to reduced evapotranspiration on the south faces. Although only a small reduction in the air thermal time during spring existed between the north and south facing sites (620 N and 630 S m a.s.l. respectively), there was more energy for evapotranspiration on the north side due to solar radiation hitting the ground/pasture surface at a higher incident angle. The lower incident sun angle on the south-facing site at 630 m a.s.l contributed to a 6-8 week delay in getting the soil temperatures above the mean air temperature compared to the north-facing site at 620 m a.s.l (Power et al. 2006).

The most notable influence of altitude on the sunny face sites of this study was the observed dominance of *T. glomeratum* over the other species at 450 and 620 m a.s.l., (Figure 2.4). Total air thermal time from mid-August to December at 450 m a.s.l. was 1440 ºCd, and declined at 100 ºCd per 100 m of elevation. At the higher sunny sites (750 and 910 m a.s.l.), all annuals except *T. glomeratum* were present. *Trifolium subterraneum* was dominant at 750 m a.s.l., while *T. dubium* and *T. striatum* were present on all sunny aspect sites and were unaffected by altitude. *Trifolium arvense* presence increased with altitude. Thermal time at 910 m a.s.l. was <1000 ºCd and indicated mature seed production of some annual clover species at or above 910 m a.s.l. may be limited. Annual clover cover and seed production would also suffer when a dry autumn causes late germination followed by an early onset of summer drought.
2.3 Nitrogen fixation

Nitrogen (N) fixation of perennial clover species in typical NZ hill country pastures has been investigated, with mean annual amounts of N fixation having been measured at hill sites in the Manawatu (Ballantrae Research Station) and in Northland at Kaikohe by Hoglund et al. (1979). Rates of N fixation ranged from 34 kg N/ha/year on browntop (*Agrostis capillaris*)-dominant Manawatu hill sites, to 342 kg N/ha/year at the warm and humid Northland site. However, the measurement of N fixation by annual clovers in NZ has been sporadic and limited in dry environments (Boswell et al. 2003a), with minimum information on their N-fixation role (Scott 2003).

Sulphur deficiency has a strong negative influence on symbiotic N fixation by *Trifolium subterraneum* (Shock et al. 1984). These authors showed N fixation was strongly enhanced by the addition of S fertiliser. Boswell et al. (2003b) found N fixation by *T. arvense* responded positively to S fertiliser application during a favourable rainfall year, in which spring and early summer rainfall was above average at Tara Hills, an inland semi-arid (539 mm rainfall/year) site near Omarama in the North Otago region of NZ. Calculated N fixation (clover N uptake minus maximum N uptake by non-legumes) ranged from 35–102 kg N/ha depending on the form of S fertiliser applied. Gypsum resulted in 35 kg fixed N/ha, while granulated ground S, S-bentonite 70% S, and S-bentonite 85% S resulted in 84, 67, and 102 kg fixed N/ha respectively.

Boswell et al. (2007) measured symbiotic N fixation of *T. arvense* at two sites in the semi-arid zone (<600 mm rainfall/year) of the Mackenzie Basin region of NZ, concluding that N fixation rates were highly variable across the mid to lower altitude (850–530 m a.s.l.) high country landscape because the distribution of *T. arvense* was variable. Variability in *T. arvense* abundance was attributed to localised distribution of seed banks in soil, differences in rainfall between years on the effect of summer moisture, and variations in favourable microsites for germination; depleted vegetation sites with sufficient bare ground with favourable soil moisture allowing colonisation. Thus, Boswell et al. (2007) stated that annual fixed N amounts were dependant on *T. arvense* occurrence and summer rainfall levels. Favourable growing seasons (~120 mm rainfall in December) resulted in approximately 3 times the amount of N fixation compared to average (~50 mm) rainfall seasons. At locations of abundant clover, ~3 kg fixed N/ha is expected in an average rainfall year and ~11 kg fixed N/ha in a favourable (wetter)
rainfall year. Following application of 25 kg/ha of S fertiliser in a favourable year, N fixation by \textit{T. arvense} increased $5^{1/2}$ times.

### 2.4 Rhizobia

Boswell et al. (2003a) stated that no research had been conducted on the specific rhizobia requirements of the four adventive annual clovers in New Zealand. In situations where appropriate rhizobia organisms are not present in the soil, it is very important to supply the appropriate rhizobia to particular legumes in order to achieve effective legume nodulation for N fixation (Carter 1984). Greenwood & Pankhurst (1976) reported that clover rhizobia are widespread in NZ soils and rhizobia effective with annual clovers are the norm in drier soils. However, a recent study investigating the effectiveness of commercial inoculant rhizobia on forage legumes (Moot pers. comm.) found that a widely used inoculant (Group C, for special clovers) failed to establish active N-fixing clover plants following inoculation of seed (D.J. Moot unpublished). Further research is required in the area of rhizobia in NZ soils, specifically around rhizobia requirements and associations with adventive annual clover species.

### 2.5 Agronomy of adventive annual clover species

#### 2.5.1 Productivity/growth/dry matter production/biomass

Boswell et al. (2003a) reported that information regarding adventive clover biomass productivity is limited in New Zealand, partly because annual clovers are expected to grow in suboptimal conditions for \textit{T. repens} production. Boswell et al. (2003b) recorded the maximum biomass of \textit{T. arvense} in field measurements within NZ, at 3300 kg DM/ha. However, these measurements were made at discontinuous patches of dense \textit{T. arvense} in an abnormally moist growing season in a semi-arid environment (<600 mm/year) near Tara Hills, Omarama. The biomass of \textit{T. arvense} recorded was in response to a 25 kg S/ha fertiliser treatment in the form of S-bentonite 85% S. Other \textit{T. arvense} recorded biomass values were 1490, 2800, and 2360 kg DM/ha, in response to 25 kg S/ha applied in the form of gypsum, granulated ground S, and S-bentonite 70% S respectively.
Brock (1973) reported *T. dubium* dry matter production ranged from 3130 to 5100 kg DM/ha in a pure sward at Palmerston North (while *T. repens* ranged from 4790 to 8290 kg DM/ha). In a study comparing the productivity of *T. dubium* and *T. glomeratum* with *T. repens* in the lower North Island, Williams et al. (1980) found *T. dubium* yielded 77% of *T. repens*, while *T. glomeratum* yielded 90% of *T. repens*. Under optimal conditions in North Island hill pastures (sunny NW slope during spring), Lambert et al. (1986) found *T. dubium* produced approximately 70% of the biomass of *T. repens* at the same site and time.

Dodd & Orr (1995) measured, in an outdoor experiment using soil cores of low fertility hill soil, the equivalent of 850 kg DM/ha from *T. arvense*, compared with 3100 kg DM/ha from *T. subterraneum*. In a similar observation of dry matter production comparison between annual clovers, Blair & Cordero (1978) found *T. glomeratum* produced 25% less biomass than *T. subterraneum* when grown in pot trials. Thus, despite the four adventive legumes being widely distributed across South Island hill and high country, there is limited information on their DM production, and hence limited information on their significance for farming systems. Furthermore, little data exists on how DM production responds to variation in soil moisture and defoliation regime.

### 2.6 Flowering and seed production - Phenology

Palmer (1972) reported that the time of flowering in *T. arvense* varied considerably between plants, both from within a collection site and between sites. These differences could not be related to environmental differences between the collecting sites. Boswell et al. (2003a) stated that in a semi-arid environment, peak flowering of *T. arvense* occurs in November, though the species can flower from August to May depending on habitat and season (Table 2.2).

Populations of *T. arvense* have been found to be self-compatible and capable of inbreeding, an advantage for plant survival in harsh conditions (Saleem & Gliddon 1989). Similarly, Palmer (1972) showed that *T. arvense* reproduction was predominately by self-pollination and between closely related individual plants. This is in contrast to *T. repens* and *T. pratense*, which were obligate outbreeders between populations of plants (Saleem & Gliddon 1989). *Trifolium dubium* produces yellow flowers from October to June depending on habitat and season, with peak flowering occurring in November in a moist environment (Table 2.2). *Trifolium glomeratum*
flowering was observed as peaking in late November 2002, after ‘Denmark’ *T. subterraneum* peak flowering at Ashley Dene, a lowland site of stony silt loam soil near Lincoln, Canterbury (R. Lucas unpublished data). No literature is reported regarding flowering time of *T. striatum* in New Zealand environments.

### 2.7 Nutritional value

The presence of tannins in herbaceous legumes occurs only in a few species. They have been found present in *T. arvense* and *T. dubium*, as flavolans (condensed tannins) by Sarkar et al. (1976). However, flavolans were not found in the leaves of *T. pratense* and *T. repens*. All the flavolans found by Sarkar et al. (1976) were proanthocyanidins. These tannins produce astringency in plants and may reduce the palatability of plant material to grazing animals (Boswell et al. 2003a). This may inhibit grazing (Scott et al. 1995) and thus increase the successful seed production by the plant.

In a study looking at the grazing preference of sheep on both developed and undeveloped grassland at a high country site, Hughes (1975) found that *T. arvense* was a preferred plant of Merino sheep in grazed tussock grassland. In addition, Reddiex (1998) showed that *T. arvense* was also a species preferred by rabbits in the same Mackenzie Basin, South Island environment. Both these studies however, measured preference of plant species by examining the frequencies of plant fragments in dung. This gave little idea of the severity of grazing on plants (Boswell et al. 2003a).

Further information on grazing preference and diet selection by sheep of these adventive clover species would be useful for fostering a greater understanding of the ecology of these plants, e.g. how these plants survive in grazed high country environments, and does grazing preference play a significant role in viable seed production through providing protection from herbivory (grazing). In addition, there is limited information on the nutritional value parameters of these adventive annual clover species, such as metabolisable energy (ME) value, % N, crude protein and soluble carbohydrate concentration, and how these values change in these species with plant development. Such information is valuable in assessment of the feeding value of these forages.
2.8 Management

2.8.1 Soils, nutrients - Response to fertilizer

Much research has been conducted investigating white clover (Trifolium repens) dry matter and growth responses to phosphorus soil fertility in New Zealand (Caradus & Snaydon 1986; Caradus et al. 1995). In contrast, little research has been conducted looking at the growth and dry matter production of naturalized adventive annual clovers (Brock 1973). It is essentially unknown how hill country legumes such as cluster, haresfoot, suckling and striated clover respond to key factors limiting growth in an extensively grazed hill/high country environment; namely, low or variable phosphorus and sulphur fertility (Dodd & Orr 1995).

Soil plant-available nitrogen levels, sourced from pasture legumes, have been positively associated with long-term fertiliser (phosphorus and sulphur) use in NZ hill country (Moir et al. 1995; 1997). Scott (2003) stated that though naturalised annual clovers respond to fertiliser, there is minimum information on how they might be incorporated into a general pasture development strategy at probably low or moderate fertiliser regimes.

In addition, recent N fertilizer trials (Gillingham et al. 2004; Smith et al. 2004) have suggested that feed production in lower altitude hill and high country is dependent on nitrogen inputs. With the suite of adventive annual clovers that are adapted to the dry and semi-arid grasslands in New Zealand (Boswell et al. 2003), and hill and high country areas often being deficient in N (Haynes & Williams 1993), more knowledge, both ecological and agronomic regarding such naturalized, adventive annual clovers, is needed and would be beneficial to land managers and farmers of hill and high country areas towards improving productivity and sustainability of these grassland/rangeland ecosystems; for example, knowledge about how these naturalised adventives respond to added phosphorus into the plant-available soil pool. By definition, annual clovers release N into the environment every year, via decomposition of plant matter and symbiotic N fixation. These pulses of N into a N-deficient system via increased plant populations of these clovers as a result of P (and/or S) additions may be beneficial to such an agroecosystem.

The soil of NZ hill and high country environments are traditionally low in plant-available P and S, (Moir et al. 1995). These two macronutrients are important for pasture legume growth and
development, especially with legumes having a greater requirement for P than grass species in the soil (Caradus 1980).

2.8.2 Grazing Management

Ates et al. (2006) conducted an experiment that investigated the effect of grazing on subterranean clover production in tall fescue *Festuca arundinacea* / *T. subterraneum* pastures in a dryland environment at Ashley Dene in Canterbury. Sheep stocking rate treatments were low (10) and high (20) ewes and their twin lambs per hectare over 46 days in spring 2005. The authors reported 62% fewer *T. subterraneum* burrs/m² under the high compared to the low stocking rate. Under both high and low stocking rates, inadequate seed production resulted in poor seedling numbers in the following autumn (285 and 223 seedlings/m² at the low and high stocking rate, respectively), well below the recommended target numbers of 500–1000 seedlings/m² (Smetham 2003).

Ates et al. (2006) suggested that these stocking rate results obtained from their experiment on the Canterbury Plains are also relevant to summer dry hill country where sunny, north facing slopes dominated by annual legumes are often grazed hard during late spring. The objective in these areas would be to accumulate pasture mass on south facing slopes where *T. repens* may contribute more than annual clovers, so that some pastures on sunny faces may be rested during the late flowering-burr production stage of subterranean clover.

At the same dryland farm used by Ates et al. (2006) *T. glomeratum* was found to dominate ryegrass *Lolium perenne* / *T. subterraneum* pastures by early summer 2003 after intensive set stocking in spring, but populations were also increased (like *T. subterraneum*) when pastures were rested early in spring to enhance flowering and seed production (Ates et al. 2008). Apart from these reported effects of intensive set stocking and resting in spring on *T. glomeratum* at Ashley Dene, little is known about the effects of grazing animals and their management on the spread and productivity of adventive annual clovers (Boswell et al. 2003a). In this context, it will be important to consider how variation in grazing intensity within hill blocks (e.g. with slope and aspect) or grazing management will affect seed production and DM production.
2.9 Summary & Research opportunities

- The four most common adventive annual clovers in New Zealand are adapted to sub-humid and semi-arid environments, which often have low natural fertility.
- Adventive annuals persist in areas and locations of regular soil moisture deficiency; summer dry environments.
- Differences in thermal time accumulation, due to differences in altitude on hill slopes and the aspect of hill slopes, influence the distribution of adventive annual clovers in a semi-arid high country grassland hill slope environment.
- There is limited information on the dry matter productivity, reproductive performance (population dynamics), nutritional value, N fixation, fertiliser response, and grazing management of adventive annual legumes persistent in hill/high country pastoral environments in New Zealand.
- Grazing preference and diet selection of adventive annual clovers by grazing animals is yet to be investigated in New Zealand.
Chapter 3

Influence of environmental factors on the abundance of naturalised annual clovers in the South Island hill and high country


3.1 Abstract

The abundance of four naturalised annual clovers (striated, cluster, suckling, haresfoot) and two sown clovers (subterranean clover and white clover) was investigated in relation to topographical, soil fertility and management factors on two contrasting hill/high country stations in the South Island: Glenfalloch in inland Canterbury (1665 mm annual rainfall), and Mt Grand in Central Otago (703 mm annual rainfall). Site surveys were conducted in three hill blocks per farm, with measurements of grassland species cover, slope, aspect, grazing intensity, soil fertility, soil depth, and altitude, made within quadrats along three transects at upper, middle and lower hill slope positions. The only naturalised clover present at Glenfalloch was suckling, whereas all were present at Mt Grand. The % cover of naturalised annual clovers was greater (30.1) than that of white clover (3.8) or subterranean clover (0.1) at Mt Grand. The % cover of white clover decreased with increasing altitude. The % cover of striated and cluster clover was greatest on sunny aspects, while white clover % cover was greatest on shady aspects. White clover cover increased and striated clover cover decreased with increasing available soil phosphorus. Naturalised annual clovers exhibit regeneration and persistence strategies that allow them to regenerate and grow in dry hill/high country pastures.

Keywords: altitude, aspect, Olsen P, soil moisture, Trifolium striatum, T. glomeratum, T. dubium, T. subterraneum, T. repens, T. arvense.
3.2 Introduction

Sustainability of high country pastoralism, following retirement of high altitude land to improve indigenous grassland conservation and recreation outcomes, has increased the need to improve the productivity of the remaining middle to lower altitude land. An important component of pastoral intensification is to increase legume abundance so as to provide increased feed and nitrogen inputs to nitrogen deficient, summer dry hill country grassland. However, the establishment and persistence of sown legume species such as subterranean clover (*Trifolium subterraneum*) and white clover (*Trifolium repens*) is often limited (Knowles et al. 2003; Power et al. 2006). This is in contrast to the common presence of other naturalised and unsown legumes, such as cluster clover (*Trifolium glomeratum*), haresfoot trefoil (*T. arvense*), striated clover (*T. striatum*) and suckling clover (*T. dubium*) (Power et al. 2006) that may be more suited to the microclimates that exist on these hill slopes (Boswell et al. 2003). However, few data exist on the distribution of these naturalised annual species in New Zealand hill country. As part of a wider study of their ecology, the distribution/abundance of these naturalised legumes on two contrasting, climatically different high country farms was surveyed.

The objective of the study was to quantify the abundance of six clover species (cluster, haresfoot, striated, suckling, subterranean and white) in relation to topography, soil fertility and management on extensive grazing blocks on two hill/high country farms in the South Island. The study also identified ecological factors influencing the distribution of these naturalised annual clovers in the variable climatic zone of the South Island hill and high country.

3.3 Methods

3.3.1 Location

Six hill country grazing blocks (paddocks ranging in size from 65 to 100 ha plus) were surveyed during late spring/early summer (December 2008 and January 2009) on two climatically different hill/high country farms, namely Mt Grand Station in Central Otago, and Glenfalloch Station in Canterbury (Table 3.1).
3.3.2 Site survey procedure

The percentage cover of each legume was visually estimated in three hill paddocks per farm. The cover of clover species, shrubs, grasses (non-tussock), and tussocks was assessed in three 5 x 5 m quadrats laid out at intervals along 200 m transects positioned in the top, middle and bottom of the slope in each hill paddock. A total of 50 quadrats (29 at Mt Grand and 21 at Glenfalloch) were surveyed. For each quadrat, local grazing intensity as indicated by visual assessment of pasture height (lax: >3 cm, intense: <3 cm), angle of slope of the quadrat to the nearest 5°, altitude (m) and aspect (full sun: NW to NE; moderate sun: NE to SE combined with SW to NW; and shady: SE to SW) were recorded. In addition, within each quadrat, depth to rock in the top 30 cm of soil was measured using a steel rod, along with soil fertility via the collection of ten soil cores (7.5 cm depth) which were bulked and analysed for pH$_{\text{H}_2\text{O}}$ (water: soil ratio 2.5:1; Blakemore et al., 1987), Olsen P (Olsen et al. 1954) and sulphate S (Searle 1979). Transects ranged in altitude from 500 to 900 m a.s.l.

3.3.3 Analysis

The percentage cover of each legume in each quadrat was used as the response variable in fitting a multiple logistic regression model using generalised linear models (Crawley 1993) with GenStat Release 12 (VSN International 2009). Due to the low abundance of annual and perennial legumes at Glenfalloch, this analysis was restricted to the Mt Grand data. A normal probability distribution with an identify link function was specified. A maximal model using nine predictor variables was constructed. The predictor variables were altitude, aspect, grazing intensity, Olsen P, perennial grass, slope, soil depth, sulphate S and soil pH. The minimum adequate model was found by deleting variables with no significant effect from the maximal model, leaving those factors whose deletion caused significant effects. Control of the model was manual at all stages.
3.4 Results

Table 3.1 Summary of climatic, topographical, and soil characteristics and mean bare ground and vegetative cover (%) on the two high country survey farms from December 2008 to January 2009 (late spring/early summer).

<table>
<thead>
<tr>
<th>Station</th>
<th>Mt Grand</th>
<th>Glenfalloch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Otago</td>
<td>703</td>
<td>1665</td>
</tr>
<tr>
<td>Canterbury</td>
<td>5.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Mean Annual Rainfall (mm)</td>
<td>188</td>
<td>345</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>172</td>
<td>370</td>
</tr>
<tr>
<td>Winter</td>
<td>3.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Spring</td>
<td>160</td>
<td>360</td>
</tr>
<tr>
<td>Summer</td>
<td>15.5</td>
<td>13</td>
</tr>
<tr>
<td>Altitude Range of survey area (m)</td>
<td>547-876</td>
<td>685-925</td>
</tr>
<tr>
<td>Soil properties</td>
<td>17.6</td>
<td>11.4</td>
</tr>
<tr>
<td>pH&lt;sub&gt;0.2&lt;/sub&gt;</td>
<td>5.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Olsen P (ug/mL)</td>
<td>15.5</td>
<td>13</td>
</tr>
<tr>
<td>Sulphate S (ug/g)</td>
<td>17.6</td>
<td>11.4</td>
</tr>
<tr>
<td>% Cover</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bare ground</td>
<td>13.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Tussock</td>
<td>1.1</td>
<td>9.2</td>
</tr>
<tr>
<td>Shrub</td>
<td>3.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Forbs/Herbs</td>
<td>3.6</td>
<td>14.1</td>
</tr>
<tr>
<td>Grass (non-tussock)</td>
<td>26.1</td>
<td>55.6</td>
</tr>
<tr>
<td>Perennial</td>
<td>30.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Annual</td>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>Perennial Legume (sown)</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>White clover</td>
<td>3.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Red clover</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Lotus pedunculatus</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Annual Legume</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Cluster clover</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td>Haresfoot clover</td>
<td>5.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Striated clover</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Subterranean clover</td>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>Suckling clover</td>
<td>4.9</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3.1 summarises the regional difference between Mt Grand and Glenfalloch in terms of annual rainfall, soil properties, and botanical composition (% cover). Total legume abundance was 34.2% at Mt Grand and 9.4% at Glenfalloch (Table 3.1). There were more annual legume
species present at the lower rainfall site of Mt Grand, with only suckling clover present at Glenfalloch (Table 3.1). At Mt Grand, striated clover was the most abundant annual legume, followed by cluster and suckling clover, with very little haresfoot and subterranean present ($P<0.05$ from ANOVA of annual legume data; Table 3.1). More sown perennial legume species were evident at Glenfalloch than Mt Grand, with white clover the dominant perennial legume (Table 3.1). Total grass cover was similar at both sites, although annual grasses were more abundant at Mt Grand than Glenfalloch (Table 3.1).

Multiple regression analyses of the % cover of legume species at Mt Grand showed that there was a significant effect of altitude for white clover, aspect for striated and cluster clover, and plant-available soil phosphorus (Olsen P) for striated and white clover (Table 3.2). There were no significant effects detected for other species and predictor variables (Table 3.2).

Table 3.2 Significance ($P$) values of predictor variables from multiple regression using generalized linear models for % cover of naturalised annual clovers and white clover % cover at Mt Grand Station, Central Otago, (*$P<0.05$; **$P<0.01$).

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>d.f.</th>
<th>Clover species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>striated</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>Aspect</td>
<td>2</td>
<td>0.002 **</td>
</tr>
<tr>
<td>Grazing Intensity</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td>Olsen P</td>
<td>1</td>
<td>0.018 *</td>
</tr>
<tr>
<td>Perennial Grass (%)</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>Slope</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>Soil Depth (cm)</td>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td>Sulphate S</td>
<td>1</td>
<td>0.68</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>0.36</td>
</tr>
</tbody>
</table>

The % cover of white clover showed a general decline with increasing altitude, although the relationship was not strong ($R^2=0.2$, Figure 3.1). White clover cover decreased from a mean of 19% at 550 m to a mean of 5% at around 900 m (Figure 3.1). The abundance of striated and cluster clover was highest on full sun aspects, intermediate on moderate sun aspects and lowest on shady aspects (Table 3.3). The % cover of striated clover decreased and white clover increased with increasing available soil P, although the relationship was not strong ($R^2=0.1$, Figure 3.2a, and $R^2=0.02$, Figure 3.2b).
Figure 3.1 The influence of altitude (m) on the cover (%) of white clover at Mt Grand Station, Central Otago, December 2008.

Table 3.3 Mean clover species cover (%) in relation to hillside aspect of three extensive hill country grazing blocks at Mt Grand Station, Central Otago, in late spring/early summer (December 2008), SE = standard error of mean.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Striated</th>
<th>Suckling</th>
<th>Cluster</th>
<th>Haresfoot</th>
<th>White</th>
<th>Sub</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate Sun¹</td>
<td>21.6</td>
<td>5.4</td>
<td>4.9</td>
<td>0.0</td>
<td>3.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Full Sun²</td>
<td>31.2</td>
<td>5.0</td>
<td>9.1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.00</td>
</tr>
<tr>
<td>Shady³</td>
<td>2.0</td>
<td>7.3</td>
<td>0.2</td>
<td>0.3</td>
<td>7.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Max S.E.</td>
<td>5.9</td>
<td>1.3</td>
<td>2.0</td>
<td>0.2</td>
<td>2.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

¹Moderate Sun: NE to SE combined with SW to NW  
²Full Sun: NW to NE  
³Shady: SE to SW
Figure 3.2 The influence of plant-available soil P (ug/mL) on the cover (%) of (a) striated clover and (b) white clover at Mt Grand Station, Central Otago, December 2008.
3.5 Discussion

3.5.1 Difference in clover species abundance between sites

There was a distinct difference in the richness and abundance of clover species between the two stations. The drier Mt Grand station in Central Otago had a higher cover of annual clover species, but a lower cover of perennial clover species, such as white clover, than the wetter Glenfalloch station in inland Canterbury. The scarcity of naturalised annual legumes at Glenfalloch is likely to be attributed to the higher rainfall at this station leading to greater perennial grass cover and competition for annual legumes (Table 3.1). Beale et al. (1993) showed rainfall had a greater effect on annual clover species distribution than temperature in a Moroccan survey, while Dear & Cocks (1997) found the presence of summer-active perennial grasses suppressed subterranean clover seedling survival due to increased drying of the soil surface. In addition, Hepp et al. (2003) demonstrated the positive effect on clover species (white, subterranean, suckling and cluster) abundance from grass suppression with herbicide in late autumn. White clover remained at low abundance at Mt Grand despite regular oversowing. Knowles et al. (2003) reported on the limitations of white clover production and persistence in drought prone regions and stated that rainfall was the major factor affecting white clover presence.

3.5.2 Altitude

Within the altitude range 500 to 900 m there was a limited effect of altitude on the abundance of clover species, with only the % cover of the perennial legume white clover reducing with altitude (Table 3.2). This is surprising given the dominant effect of increasing altitude on lowering thermal time accumulation (Power 2006). However, cluster, haresfoot, striated and suckling clover have lower thermal time requirements for emergence and seedling development than white clover (Lonati et al. 2009). Hence, these naturalised annual clovers may germinate rapidly after autumn rains, develop a leaf canopy, and have the ability to reach an advanced stage of seedling development before the onset of metabolic-limiting low winter temperatures.
3.5.3 Aspect

Striated and cluster clover increased in % cover on sunny aspects, while white clover and suckling clover were more prevalent on shady aspects. Our results support the contention of Power et al. (2006) that the collective dominance of naturalised annual clovers on warm sunny faces (below 910 m a.s.l.) is due to reduced competition for micro-sites and growth from the perennial grass sward. On these sites, naturalised annual species appear to be able to complete their lifecycle before the onset of dry and warm conditions, typical of sunnier northern aspects (Lambert & Roberts 1976). Of note, is that suckling clover was not affected by aspect (Table 3.2 and 3.3), appearing to have a scrambling growth and regeneration strategy for a wide range of micro-sites.

White clover was also not significantly influenced by aspect in the multiple regression model (Table 3.2), although there was a trend of more white clover in shady, moister aspects. In part, the lack of an effect for white clover may reflect that the species also persists by seedling recruitment on the sunny faces, behaving in similar fashion to an annual species (Edwards, Clark & Newton 2001). Moreover, Chapman (1987) found seedling survival of T. repens in North Island summer-moist hill country (1280 mm/year) was highest on steep north-west sites and nil on flat south-west sites, a reflection of competitive stresses from surrounding vegetation.

3.5.3 Available phosphorus

Our finding that striated clover declined with increasing Olsen P has not been reported before in New Zealand. In addition, a recent glasshouse pot trial (T.M.R. Maxwell; Chapter 5) also demonstrated low dry matter response of striated clover to increasing levels of available phosphorus in high country soil, compared with the higher dry matter responses of ‘Nomad’ white clover, ‘Mt Barker’ subterranean clover, suckling, cluster and haresfoot clovers. This result with striated clover is possibly due to its adaptation to low soil P environments (Dodd & Orr 1995). Beale et al. (1993) reported T. striatum (striated clover) as being one of four annual clover species associated with soils of very low P status, among sixteen species identified in an ecogeographic survey of agricultural zones in Morocco. White clover cover increased with increasing Olsen P in this study, as has been previously reported (Caradus & Snaydon 1986; Caradus et al. 1995; Singh & Sale 1998).
It was surprising to find no relationship between the clover abundance and grazing intensity, as other studies have shown marked impacts of grazing intensity on clover abundance. For example, Ates et al. (2006) found cluster clover to dominate ryegrass/subterranean clover pastures by early summer after intensive set stocking in spring, and that populations were also increased when pastures were spelled early in spring to enhance flowering and seed production. Our results suggest naturalised species are more tolerant of grazing, namely they have a lower grazing preference, produce large amounts of seed, and that the differences in grazing intensity were not marked enough to produce a response.

### 3.6 Conclusions and Practical Implications

- Soil moisture, as determined by annual rainfall, is probably the main factor determining the presence/absence of naturalised annual clovers in South Island hill and high country.
- Aspect was a dominant factor affecting the abundance of annual legumes with higher abundance in sunny, north facing aspects.
- Naturalised annual clovers grow in combination where the sown species, white and subterranean clover, remain at low abundance, and may contribute significantly to dry hill/high country through pasture production and nitrogen inputs.
- These legumes exhibit regeneration and persistence strategies (avoidance of grazing, high seed production, tolerance of low soil pH, S and P) that allow them to regenerate and grow on dry hill/high country pastures. These strategies could be exploited in breeding programmes for dry hill/high country pastures.

**Acknowledgements**

The Miss E L Hellaby Indigenous Grasslands Research Trust for generous funding; Evan Gibson, Farm Manager of Mt Grand Station, and Chas and Dietlind Todhunter of Glenfalloch Station willingly provided access to their properties for us to conduct research.
Chapter 4
Adventive annual clover species biomass production, botanical contribution and population dynamics in relation to spring grazing management and superphosphate input

4.1 Introduction

Grazed grassland productivity in New Zealand hill and high (>700 m a.s.l.) country is driven strongly by nitrogen (N) inputs sourced primarily from sward legumes and the associated biological N fixation (Haynes & Williams 1993). Most hill and high country soils are low in available N (White 1990). Nitrogen is the principal nutrient driving pasture growth (Chapman & Macfarlane 1985) thus increasing the legume component within the grazed sward is an inherent objective of pastoral improvement (intensification) for hill and high country grassland communities. Recent N fertiliser trials strongly demonstrate that livestock feed production in hill country and lower altitude high country is dependent on N inputs (Gillingham et al. 2004; Smith et al. 2004). Providing increased N inputs to N-deficient grassland, and increasing the nutritional value of available herbage for livestock through introducing legume species and/or enhancing overall legume abundance is the traditional model of pasture improvement in New Zealand (Bowatte et al. 2006) and is important for sustainability of grazed high country grassland (Boswell et al. 2007).

Economic return from commodities produced from high country grazing systems is under pressure following retirement of summer-grazing high altitude areas (>1000 m a.s.l.). The aim is to improve indigenous grassland conservation (preservation) and recreation resources, driven by central government policy (O’Connor 2003; Norton 2004; Barratt et al. 2006; Gillespie 2006; Brower 2008). The general trend of decreased wool prices in recent decades (Meat and Wool New Zealand Economic Service 2009) has resulted in a shift from primarily producing wool, as the main source of farm income, to store lamb and calf production in conjunction with wool as a means to increase hill and high country farm income (FAO 2009). The greater use of terminal sires for store lamb production and its associated earlier lambing dates accentuates the necessity of improved mid–lower altitude grazing areas so pastures with increased feeding value are
available to lactating ewes in spring and weaned lambs in summer. Furthermore, recent land use
conversion in lowland areas from intensive sheep and beef finishing (fattening) to dairying and
dairy grazing has forced hill and lower altitude high country areas to intensify (FAO 2009) as
fattening destinations for store lamb and cattle from hill and high country farms are being
displaced by these dairy conversions. If high country pastoralism is to remain both ecologically
sustainable and economically viable, improvement of both the productive capacity of remaining
middle and lower altitude grassland (900–300 m a.s.l.) of various hill slope and aspects, and the
current pasture management of such areas used for extensive livestock grazing in predominately
merino (fine) wool, lamb and beef production enterprises is therefore required (Maxwell et al.
2010).

Achieving greater legume abundance necessitates the application of phosphorus (P), sulphur (S)
and lime (CaCO₃) to alleviate soil fertility factors, of which low available P and S and low pH
(4.5–5.5) conditions are the major native edaphic constraints to pasture productivity, and more
specifically pasture legume productivity in NZ hill and high country areas (Edmeades et al.
1984a; Moir & Moot 2010). The soil on ~500,000 ha of farmed high country has low soil pH and
possibly high aluminium (Al) levels, which along with low available soil P and S, may
collectively limit establishment and persistence of sown pasture legumes (Moir et al. 2000; Moir
&Moot 2010).

Soil N input from pasture legumes is strongly influenced by soil P fertility (Moir et al. 2000) with
greater annual biological soil N inputs resulting from larger sward legume content in response to
higher soil P fertility in hill country (Gillingham et al. 2008). Phosphorus requirements for
optimum growth of traditionally sown pasture legumes are generally greater than that of grass
species (Caradus 1980). As such, highly productive pasture legume species like *Trifolium repens*
L. are generally adapted to high fertility soil conditions and their performance is comparatively
poor in infertile and/or acid dryland soil conditions (Haynes & Williams 1993; Moir et al. 1997).
Soil sulphur amendment requirements for greater pasture legume growth in hill and high country
soils rise with increasing distance from the coastline in New Zealand, with widespread S
deficiencies on South Island soils (Edmeades et al. 2005).

Improved legume-based pastures are especially important within summer dry (<800 mm rainfall
per annum) eastern regions of New Zealand, where seasonal soil moisture deficits caused by
evapotranspiration exceeding summer rainfall (Brown & Green 2003) constrains potential pasture production. In such dryland environments maximizing pasture productivity and quality during the period of reliable soil moisture (autumn to mid-spring) is essential (Gillingham et al. 2003). Establishment and persistence of broadcast-sown legume species (white clover *T. repens* L. and subterranean clover *T. subterraneum* L.) is often limited (Knowles et al. 2003; Power et al. 2006) in hill and high country grassland agroecosystems. These encompass dry eastern areas within the North Island East Coast, Gisborne, Hawke’s Bay, Wairarapa, Marlborough, Canterbury and East Otago regions, including inland areas of South Canterbury and Central Otago, despite repeated oversowing particularly with *T. repens* (Power et al. 2006).

Hill and high country slopes of north-facing (sunny) aspects commonly experience late spring–summer soil moisture deficits due to a combination of climatic and topographical (physical land) factors which determine the available soil moisture regime; namely low seasonal rainfall from November to April (Knowles et al. 2003), and reduced rainfall effectiveness on sloping land. Specifically, South Island hill and high country, owing to low and extended winter temperatures, is epitomized by a short growing season that is often limited by soil moisture (Moir & Moot 2010). In comparison to flat land pasture areas, the sloping nature of hill country land causes reduction in pasture production potential, as significant rainfall is lost via runoff, thus reducing rainfall infiltration and limiting soil moisture content (Bircham & Gillingham 1986). In addition, water storage capacity of hill country soil is generally lower due to variable topsoil depth that is more often shallow, making localised soil moisture levels very dependent on the frequency of rainfall (re-wetting) events rather than solely total rainfall amount in spring–summer (Bircham & Gillingham 1986). These climo-topographical factors cause the characteristic dryland edaphic conditions of annual moisture deficits manifested as soil moisture stress from late spring–summer (Williams et al. 1990). These conditions are detrimental to the production, survival and thus persistence of the most widely sown perennial pasture legume, white clover *T. repens* (Knowles et al. 2003; Power et al. 2006) which phenologically exhibits optimum growth and stolon propagation in summer when soil moisture stress is greatest.

Contrastingly, other naturalized but unsown and adventitious annual legumes; haresfoot trefoil *T. arvense* L., suckling clover *T. dubium* Sibth, cluster clover *Trifolium glomeratum* L. and striated clover *T. striatum* L. are commonly present in summer dry zones, and specifically on north-facing slopes of hill and high country areas (Boswell et al. 2003a; Power et al. 2006; Maxwell et
Most adventive annual clover species are considered small in stature, and associated generally with lower soil fertility conditions and hence lower pasture productivity (Scott 2003). The main attribute of these clover species within New Zealand pastoral agriculture, as with sown pasture legume species, is improving overall pasture quality and increasing available N in the soil for the benefit of other herbaceous species (grasses and herbs) in the pasture community via their N-fixation capacity. Information as such regarding biomass productivity of these four adventive clover species is limited in New Zealand however, partly because they are expected to grow in conditions considered suboptimal for *T. repens* production (Boswell et al. 2003a). Little research has been conducted looking at the growth, dry matter production, and nutrient (P and S) and lime response of adventive annual clover species either in climate-controlled studies or field trials. Detail on the grazing management response, level of dry matter (DM) production, and N-fixation of these species is limited.

Boswell et al. (2003b) recorded maximum biomass of 3300 kg DM/ha from dense, discontinuous patches of *T. arvense* during an abnormally moist growing season at Tara Hills, Omarama, a semi-arid (540 mm annual rainfall) environment of Central Otago. *Trifolium dubium* dry matter production ranged from 3130 to 5100 kg DM/ha in a pure sward at Palmerston North receiving 883 mm annual rainfall and irrigation at times of maximum water stress in late summer and autumn, while neighbouring pure swards of *T. repens* cv. Huia ranged from 4790 to 8290 kg DM/ha, (Brock 1973). In a study comparing the productivity of *T. dubium* and *T. glomeratum* with a relatively short-lived, cool season-active experimental variety of *T. repens* at three sites across the lower North Island (Palmerston North and Bulls in the Manawatu, and Wimbledon in Hawke’s Bay), Williams et al. (1980) found *T. dubium* yielded 77% of *T. repens* at the Hawke’s Bay site, while *T. glomeratum* yielded around 90% of *T. repens* at all sites. At Ballantrae in southern Hawke’s Bay (steep, low fertility, moist hill country; 1280 mm mean annual rainfall), Lambert et al. (1986) found *T. dubium* produced approximately 70% of *T. repens* biomass on a sunny NW slope during spring. Dodd & Orr (1995) measured, in an outdoor experiment using soil cores of low fertility hill soil, the equivalent of 850 kg DM/ha from *T. arvense* compared with 3100 kg DM/ha from *T. subterraneum*. In a similar observation of DM production comparison between annual clover species, Blair & Cordero (1978) found *T. glomeratum* produced 25% less biomass than *T. subterraneum* when grown in pot trials.
There is evidence to suggest that grazing deferment positively influences grass species productivity and persistence because of increased natural reseeding (L’Huillier & Aislabie 1988; McCallum et al. 1991; Boom & Sheath 1990). Studies on increasing pasture legume abundance via natural reseeding are few (Lowther et al. 1992). Increasing the abundance of a pasture species in continuously sheep-grazed pastures involves techniques such as encouraging seed production and seed fall through deferred grazing in spring and summer (Edwards et al. 2005). There is no literature on the effect of grazing deferment on natural reseeding of annual clover species (seed production and seedling recruitment) in South Island summer dry hill and high country. Ates et al. 2006; 2008; Ates 2009 investigated the influence of varying spring closing dates at low and high stocking rates on T. subterraneum seed production and subsequent seedling recruitment in a lowland summer dry Canterbury environment. Ates et al. (2006) reported T. glomeratum was not significantly influenced by different spring grazing management regimes (high and low grazing pressure) imposed at a dryland (<700 mm) Canterbury Plains site in predominately sub clover T. subterraneum and cocksfoot Dactylis glomerata based pastures.

Thus, despite these four adventive annual clover species being widely distributed across South Island hill and high country (Hoglund 1990; McIntosh & Allen 1998; Boswell et al. 2003a; Rose & Frampton 2007; Maxwell et al. 2010), there is limited information on their DM production and response to superphosphate, and scarce literature on their response to different grazing management regimes. Hence, information on their significance for farming systems, particularly those existing in low-input, hill and high country dryland areas, is required.

The effect of grazing (spring closure) and soil fertility (P and S) management on the botanical composition, dry matter production, and population dynamics of these four clover species was investigated in a two year field study at a summer dry (<800 mm mean annual precipitation) hill and high country site (680–700 m a.s.l.) on an extensive sheep and beef high country station in Central Otago, South Island, New Zealand. The objective of this field study was to quantify the effect of early and late spring grazing closure versus continuous grazing in spring, and low and high superphosphate (P and S) application on the sward composition, herbage dry matter and seed production, and subsequent seedling recruitment of these four adventive annual clover (pasture legume) species growing as naturalised populations within an upland pasture community in an extensively grazed hill block typical of dryland South Island hill and high country.
4.2 Materials and Methods

4.2.1 Site Description

The experiment was conducted at the Lincoln University high country farm, Mt Grand Station, a 2131 ha commercially run hill and high country property located south east of Lake Hawea in the Central Otago district on an eastern wall of the Upper Clutha Basin in the South Island (44°38'01.93” S; 169°19'42.89” E; altitude 380–1380 m a.s.l.). Long-term mean annual rainfall (60 years) is 703 mm, with high annual and monthly variability (Figure 4.1).

![Annual rainfall (mm)](image)

**Figure 4.1** Annual and monthly rainfall during the study period of 2008–2010 at the mid-altitude (680–700 m a.s.l.) high country field site on Mt Grand Station, Lake Hawea, Central Otago, New Zealand. NB autumn (March–May), winter (June–August), spring (September–November), and summer (December–February).

Mean winter temperatures were 4.2°C (day) and 2.7°C (night). Mean summer temperatures were 15.1°C (day) and 11.7°C (night), with a minimum mean of 1.5°C in June and a maximum mean of 15.5°C in February during the experimental period from October 2008–May 2010.
The field trial was situated on a north-facing (sunny) hillside of moderate to steep (20–30°) slope, ranging in altitude between 680–700 m a.s.l. on an Arrow steepland soil (Pallic/Yellow Grey Earth; USDA classification Fragiudalf) with schist and loess parent material (Duncan et al. 1997; Power et al. 2006). The trial site had moderately low soil pH and plant-available P and S levels (Table 4.1). The trail site was located within a 65 ha hill block called Broadspur known to have established populations of the four adventive annual clover species, with T. repens and T. subterraneum being present alongside an array of annual and perennial grass species in the grassland community (Table 4.2). Shrub species present at the site were sweet briar Rosa rubiginosa L., matagouri Discaria toumatou and kanuka Kunzea ericoides (Plate 4.1).

Table 4.1 Soil chemical properties of the mid-altitude (680–700 m a.s.l.) high country field trial site at Broadspur hill block, Mt Grand Station, Central Otago, New Zealand, August (winter) 2008.

<table>
<thead>
<tr>
<th>Soil chemical properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.5</td>
</tr>
<tr>
<td>Olsen P</td>
<td>18 mg kg⁻¹</td>
</tr>
<tr>
<td>Sulphate-S</td>
<td>9 mg kg⁻¹</td>
</tr>
<tr>
<td>Organic-S</td>
<td>7 (mg kg⁻¹)</td>
</tr>
<tr>
<td>Exchangeable K</td>
<td>8 (cmolₑ kg⁻¹)</td>
</tr>
<tr>
<td>Exchangeable Mg</td>
<td>18 (cmolₑ kg⁻¹)</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>5 (cmolₑ kg⁻¹)</td>
</tr>
<tr>
<td>Exchangeable Na</td>
<td>1 (cmolₑ kg⁻¹)</td>
</tr>
</tbody>
</table>
Plate 4.1 Field site showing mid spring (November 11\textsuperscript{th} 2008) pasture and resident shrub species (sweet briar \textit{Rosa rubiginosa} L., matagouri \textit{Discaria toumatou} and kanuka \textit{Kunzea ericoides}) dispersed within the mid altitude (680–700 m a.s.l.) high country pasture during the first year of sampling, and continuously grazed main plots (5 x 5 m) with steel exclosure cages at Broadspur hill block, Mt Grand Station, Central Otago, New Zealand.

The grazing management regime in Broadspur hill block for the duration of the 2 year field experiment followed standard grazing practice for sunny (north-facing) aspects of the farm, whereby continuous grazing with 300 pregnant (single lamb) Merino ewes (4.6 SU/ha) occurred from the start of spring growth and lambing in late September, through to February (late summer) when grazing ceased until late April (mid-autumn). From April to mid-July (autumn through to mid-winter) the hill block was grazed by 300 ewes and 4 rams, then spelled again from mid-July before grazing resumed with pregnant ewes again in mid-September at a stocking rate of 4.6 SU/ha, to coincide with the comparatively late start of spring pasture growth (around 20\textsuperscript{th} September). In addition, rising two year-old, store beef cattle of mixed-breed (trading heifers and steers) were also stocked from April to September to assist with control of pasture quality by grazing down areas of accumulated standing dead matter (tag).
4.2.2 Experimental design and Treatments

The experiment was a four (grazing management) by two (superphosphate) factorial split plot design, with spring grazing management treatments as main plots and superphosphate application treatments as split plots. There were four replicates (blocks) making 36 plots in total. Main plots were 5 x 5 m and split plots 2.5 x 5 m in dimension respectively. Main plot treatments were continuous grazing (standard grazing management as described above, with and without wire netting), early-mid spring closure (grazing ceased in October), and late spring closure (grazing ceased in November). Split plots were low (75 kg/ha) and high (200 kg/ha) rates of 30% sulphur superphosphate (SP) respectively; low SP being 5 kg P and 23 kg S/ha respectively, while high SP was 9 kg P and 38 kg S/ha respectively. Fertilizer was applied to respective split plot areas on 11<sup>th</sup> November 2008. Grazing exclusion in spring was achieved by fencing main plot areas with waratahs (narrow steel posts) and livestock-proof wire netting. In the first year of data collection, early-mid spring closure plots were closed to grazing on the 16<sup>th</sup> October 2008, while late spring closure plots were closed on the 11<sup>th</sup> November 2008. In the second year, early spring closure plots were shut on 18<sup>th</sup> November 2009, and late spring closure plots were closed on 27<sup>th</sup> January 2010.

The grazing management treatments imposed represent typical grazing practices that exist on a South Island hill and high country station located in a summer dry zone that experiences climatic variation, specifically annual and seasonal rainfall variation (Figure 4.1). The three grazing management treatments were designed to simulate grazing pressure all the time (continuously) in spring, and removing grazing animals either early-mid spring (early spring closure), or late in spring (late spring closure) to spell the hill block from grazing allowing general pasture accumulation and set seeding in years with abundant spring and early summer rainfall. All main plot areas excluded from grazing as part of early and late spring closure grazing management treatments were opened up to livestock in late autumn (May) in both years to allow grazing down of accumulated pasture.

4.2.3 Measurements

Dry matter (DM) and visual botanical composition (% cover) measurements were made twice in spring during the first year of data collection (November 11<sup>th</sup> and December 2<sup>nd</sup> 2008), once during the second year (November 18<sup>th</sup> 2009), and once during summer (January 27<sup>th</sup> 2009,
March 10th 2010) and autumn (May 6th 2009, May 26th 2010) of both years. Seedling measurements were made in late autumn (May) of both years. Data was collected over a two-year period to determine any carry-over effects of spring grazing closure treatments in following season (Table 4.2). Seed production measurements were made in the summer of the first year when abundant clover flowering occurred.

Table 4.2  Spring grazing management treatments imposed, relative to continuous grazing, and hypothesised carry-over effects on adventive annual clover species reproductive performance at Broadspur hill block during the two year experiment, Mt Grand Station, Central Otago, New Zealand.

<table>
<thead>
<tr>
<th>Spring Grazing Management</th>
<th>Carry-over effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early spring closure</td>
<td>Increased clover seed production in late spring – summer</td>
</tr>
<tr>
<td>(mid-October)</td>
<td>Greater clover seedling recruitment in following autumn</td>
</tr>
<tr>
<td>Late spring closure</td>
<td>Increased clover seed production in summer</td>
</tr>
<tr>
<td>(mid-November)</td>
<td>Greater clover seedling recruitment in following autumn</td>
</tr>
</tbody>
</table>

4.2.3.1  Dry matter production
Herbage samples were collected in spring, summer and autumn, over a two-year period from October 2008–May 2010. Herbage was harvested from within two 0.2 m² quadrats selectively placed inside each split plot area. One quadrat cut was taken of herbage accumulated within pegged-down steel cages to measure pasture growth in the absence of grazing (accumulated biomass), and one quadrat cut was taken of herbage standing outside steel cages to measure pasture growth in the presence of grazing (steady state/standing biomass) over the pasture growing season. Quadrats were placed on a site seen as representative of the overall herbage cover, within the caged area, and outside in the split plot area, at the time of sampling. Herbage was harvested to 2 cm above ground level. The resting position of pasture accumulation cages within the continuous spring grazing treatment plots was always different after each harvest event, with the new position selected being representative of the split plot area.

4.2.3.2  Botanical composition
Botanical composition of the herbage growing within plots was determined by: (i) visual cover estimates (%) of each clover species, total grasses, forbs (weeds and herbs), and dead matter
components within two 0.2 m² quadrats at each harvest time; and by (ii) DM values of individual clover species and other botanical components from herbage cuts were determined from the same quadrat-sampling sites in which visual estimates were made. Botanically sorted herbage samples were oven-dried at 70ºC for 48 hours and weighed.

4.2.3.3 Reproductive performance/Population Dynamics

Seed production was determined by: (i) collecting five seed heads from each clover species present within main plots and counting the number of seeds per head; and (ii) by counting the number of seeds from each clover species present within herbage harvested from one 0.2 m² quadrat in each split plot area during dry matter harvests in summer of the first year.

Seedling recruitment of clover species was measured each autumn over the two year sampling period by counting the number of seedlings present on 20 soil cores collected from within each subplot area using a soil core sampling tool; the dimensions of a soil core being 6.1 cm diameter by 4.5 cm depth; a soil surface area of 29.2 cm² and a soil volume of ~131.5 cm³. Twenty soil cores with an area of 29.2 cm² each gave a total area sampled of 584.5 cm², or 0.05845 m². All seedlings were counted as an estimate of autumn regeneration by the six clover species present at the site.

4.2.3.4 Statistical analysis

Mean values for % visual cover and biomass (% and kg DM/ha) of all clover species, grass (total grasses), weed, herb and dead matter components collected in the first and second year were analysed via analysis of variance (ANOVA) to determine any statistically significant differences between plant groups (clover species and the other botanical groups) and blocks (replicates). The effects of harvest time (sampling time during growing season), spring grazing treatments, soil fertility treatments, and their interactions on mean botanical composition and mean sward biomass (standing and accumulated) were statistically analysed by conducting a split split-plot ANOVA on the two year data set. The effects of spring grazing treatments and soil fertility treatments on mean seed production and mean seedling recruitment were statistically analysed by conducting a split plot ANOVA. All statistics were conducted using GenStat 13 (Lawes Agricultural Trust, Rothamsted, UK).
4.3 Results

4.3.1 Botanical composition and standing biomass

The dominant botanical components were dead matter, grass, and adventive annual clovers respectively, with weeds, sown clovers, and herbs contributing the least to the north-facing mid altitude hillside pasture sward over the two-year sampling period (Table 4.3). From November 2008–May 2010, mean total clover component (adventive and sown species) was less abundant than mean grasses component. However, the resident adventive annual clover species, collectively (AACs), contributed significantly more to the pasture sward than the sown clover species of *Trifolium repens* and *T. subterraneum*. Adventive annual clover species accounted for 97% and ~90% of the sward clover content in the first and second year respectively. In terms of the total sward, AACs accounted for ~27% and ~2% in the first and second year respectively, while sown clover species only accounted for ~0.8% and ~0.3% in the first and second year respectively (Table 4.3).

Individual clover species abundance in the first year (November 2008–May 2009) was in the order of *T. striatum* > *T. glomeratum* ≥ *T. dubium* > *T. arvense*, *T. repens* and *T. subterraneum* respectively (Table 4.3). In the second year (November 2009–May 2010) *T. striatum* was again the most abundant clover species, however all other clover species were not significantly different from each other (*P*<0.001, Table 4.3). *Trifolium arvense* was the only adventive annual clover species of small stature, not differing significantly from sown clover species (*T. repens* and *T. subterraneum*) in terms of biomass and % visual cover in both years (*P*≤0.001, Table 4.3).

Overall clover abundance at the mid altitude high country site varied greatly between the two years in which sampling occurred. Clover biomass, expressed hereafter as % and kg DM/ha, and % visual cover of total clover was greater in the first year (spring 2008–autumn 2009) when mean total clover content (all species) was 28% (689 kg DM/ha). This fell to a mean of 2.1% (58 kg DM/ha) in the second year (mid spring 2009–late autumn 2010). Similarly, mean total clover % visual cover fell from 30% in the first year to 9.6% in the second year (Table 4.3). Overall, the clover content of the sward was greatly reduced in the second year.

The dominant clover species in the mid altitude high country pasture community was *T. striatum*, being significantly greater (*P*<0.001) in biomass and % visual cover over all other clover species
(adventive and sown) in both years (Table 4.3). *Trifolium striatum* accounted for ~50% and ~80% of mean total clover biomass in the first and second year respectively. When expressed in terms of mean total sward biomass however, *T. striatum* accounted for 14% (343 kg DM/ha) in the first year and only 1.7% (47 kg DM/ha) in the second year (Table 4.3). This is a strong reflection of the scarcity of clover species present, in general, during mid-spring of the second year, which is in gross contrast to clover content at the same time in the previous year.

*Trifolium glomeratum* was the second most dominant clover species in the first year, contributing a mean of 7.2% (187 kg DM/ha) to the total sward biomass (Table 4.3). *Trifolium dubium* was the third most dominant clover species contributing 5.7% (126 kg DM/ha), (Table 4.3). *Trifolium glomeratum* and *T. dubium* accounted for 25–27% and 18–20% of mean total clover biomass respectively in the first year (Table 4.3). *Trifolium arvense, T. repens* and *T. subterraneum* were the least dominant clover species with significantly less (*P*<0.001) biomass at 0.4% each (<2% of mean total clover biomass), equating to 12.6, 15.2, and 8.9 kg DM/ha respectively (Table 4.3). In the second year however, mean biomass and % visual cover of *T. glomeratum, T. dubium, T. arvense, T. repens* and *T. subterraneum* were not significantly different from each other (*P*<0.001, Table 4.3). All these clover species collectively, other than *T. striatum*, contributed <21% to mean total clover biomass in the second year; their collective contribution the year before was nearly half (49.6%) of total clover biomass (Table 4.3). Again, this reflects the large contrast in sward clover content between the two years.
Table 4.3  Mean botanical components of the mid-altitude (680–700 m a.s.l.) summer dry high country grassland community at Broadspur hill block, Mt Grand, Central Otago, New Zealand, over a two year period from October 2008–May 2010, expressed as visual estimate (% visual cover) and biomass (% and kg DM/ha). *** , ** and * indicate a clover species or botanical component group being significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ respectively. Different letters denote means that are significantly different @ 5% level.

<table>
<thead>
<tr>
<th>Botanical components</th>
<th>Visual cover (%)</th>
<th>Biomass (%)</th>
<th>Biomass (kg DM/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 1</td>
<td>Year 2</td>
<td>Year 1</td>
</tr>
<tr>
<td>Adventive clovers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. arvense</em></td>
<td>0.4 a</td>
<td>0.1 a</td>
<td>0.4 a</td>
</tr>
<tr>
<td><em>T. dubium</em></td>
<td>5.9 b</td>
<td>0.6 a</td>
<td>5.7 b</td>
</tr>
<tr>
<td><em>T. glomeratum</em></td>
<td>6.5 b</td>
<td>0.3 a</td>
<td>7.2 b</td>
</tr>
<tr>
<td><em>T. striatum</em></td>
<td>15.8 c</td>
<td>8.0 b</td>
<td>14.4 c</td>
</tr>
<tr>
<td>Sown clovers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. repens</em></td>
<td>1.0 a</td>
<td>0.5 a</td>
<td>0.4 a</td>
</tr>
<tr>
<td><em>T. subterraneum</em></td>
<td>0.9 a</td>
<td>0.1 a</td>
<td>0.4 a</td>
</tr>
<tr>
<td>Total clover</td>
<td>30.2</td>
<td>9.6</td>
<td>28.4</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>2.1</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Grasses</td>
<td>46.8 d</td>
<td>55.6 d</td>
<td>35.5 b</td>
</tr>
<tr>
<td>Weeds</td>
<td>5.1 b</td>
<td>7.9 b</td>
<td>2.6 a</td>
</tr>
<tr>
<td>Herbs</td>
<td>1.9 a</td>
<td>1 a</td>
<td>0.1 a</td>
</tr>
<tr>
<td>Dead</td>
<td>12.0 c</td>
<td>40.8 c</td>
<td>33.4 b</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>3.1</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Grass species present: Browntop *Agrostis capillaris* L., Kentucky bluegrass *Poa pratensis* L., Annual poa *Poa annua* L., Sweet vernal *Anxanthurum odoratum* L., Tall oat grass *Arhenatherum elatius* L., Blue wheat grass *Elymus* spp., Danthonia grasses *Rytidosperma* spp., Perennial ryegrass *Lolium perenne* L., Cocksfoot *Dactylis glomerata*, Downy brome *Bromus tectorum* L., Ripgut brome *Bromus diandrus* Roth, Needle grass *Austrostipa nodosa*

1Weed and herb species present: Mouse-ear hawkweed *Hieraceum pilosella*, Hawksbeard *Crepis capillaris* L., Storksbill *Erodium cicutarium*, Sheep’s sorrel *Rumex acetosella* L.

Mean Annual Pasture biomass

<p>| | | |</p>
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<tr>
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<tr>
<td></td>
<td>2500</td>
<td>2433</td>
</tr>
</tbody>
</table>

1 Grass species present: Browntop *Agrostis capillaris* L., Kentucky bluegrass *Poa pratensis* L., Annual poa *Poa annua* L., Sweet vernal *Anxanthurum odoratum* L., Tall oat grass *Arhenatherum elatius* L., Blue wheat grass *Elymus* spp., Danthonia grasses *Rytidosperma* spp., Perennial ryegrass *Lolium perenne* L., Cocksfoot *Dactylis glomerata*, Downy brome *Bromus tectorum* L., Ripgut brome *Bromus diandrus* Roth, Needle grass *Austrostipa nodosa*
Dead matter (litter and standing matter) content within the sward showed the opposite trend to that of AACs during the two-year sampling period, with more dead matter present in the second year than the first. Mean dead matter biomass (% and kg DM/ha) was around twice as much in the second year (66.6% and 1570 kg DM/ha) than in the first (33.4% and 859 kg DM/ha), while mean % visual cover of dead matter was over three times as much (40.8%) in comparison to the first year (12%), (Table 4.3).

Grass, weed, and herb components in the sward did not differ greatly between the two years, in comparison to the variation observed for the clover component. However, less grass biomass was observed in the second year (27.3%; 730 kg DM/ha) than in the first (35.5%; 888 kg DM/ha, Table 4.3). Weed and herb biomass was fairly constant over the growing season during the two years, ranging from 62–65 kg DM/ha (~3%) and 2–11 kg DM/ha (~0.5%) respectively (Table 4.3, Figures 4.2 and 4.3).

Figure 4.2 Mean botanical components of sward standing biomass (%) at starting point (16th October 2008) and harvest dates during the study period November 2008–May 2010, from Broadspur field site, 680–700 m a.s.l., Mt Grand Station, Central Otago, New Zealand. Error bars are ± SEM.
Pasture biomass in the first year ranged between 1673–3582 kg DM/ha (Figure 4.3), averaging 2.5 t DM/ha during the spring 2008–autumn 2009 period (Table 4.3). Pasture mass in the second year ranged between 1474–3523 kg DM/ha (Figure 4.3), averaging 2.4 t DM/ha between spring 2009–autumn 2010 (Table 4.3).

4.3.2 Influence of Season

Seasonality had the greatest influence on botanical composition and pasture standing biomass at the mid altitude high country hill site (Table 4.4, Figures 4.2 and 4.3). Significant differences ($P<0.001$) between standing biomass (expressed hereafter as % of total sward mass, and kg DM/ha) and visual cover (%) for dominant botanical components (adventive annual clovers, grass, dead matter) between harvests dates in spring, summer and autumn of the first year were evident. This reflected the rise and decline of living pasture mass composed of predominately annual clover species and annual and perennial grass species through spring to autumn, and rising dead matter accumulation. Apart from mid spring (11th November 2008) and late spring/early
summer (2nd December 2008) harvests of AACs, grass and dead matter not being significantly different, midsummer (27th January 2009) and late autumn (6th May 2009) harvests were different from each other, and different from the mid spring and late spring/early summer harvests respectively (Figures 4.2 and 4.3). The three harvests in the second year (mid spring, late summer, late autumn; 17th November 2009, 3rd March, and 26th May 2010 respectively) were significantly different (Table 4.4, Figures 4.2 and 4.3).

During the first year (October 2008–May 2009) grass and AACs were equally dominant components of the sward during spring, with both increasing biomass in parallel fashion from early spring (October 16th) to mid spring (November 11th) (Figures 4.2 and 4.3). From mid spring, grass began to increase faster than AACs leading into late spring/early summer (December 2nd 2008) (Figures 4.2 and 4.3). From early summer onwards, AACs began to decrease while grass continued to increase steadily into mid-summer (January 27th 2009). Grass became dominant from late spring/early summer, before dropping sharply in late summer leading into autumn (May 6th 2009) (Figures 4.2 and 4.3). Adventive annual clovers reached peak standing biomass (38%; 758 kg DM/ha) in the mid spring–late spring/early summer period between the first and second harvest dates (Figures 4.2 and 4.3). Peak grass biomass (55%; 1816 kg DM/ha) occurred in the mid–late summer period. Dead matter began to increase steadily in late spring/early summer, continuing strongly into autumn, reaching over 80% (Figure 4.2) and exceeding 2000 kg DM/ha (Figure 4.3) at peak standing biomass. Dead matter became dominant in the sward around late summer/early autumn.

During the second year (November 2009–May 2010) AACs standing biomass was greatly reduced. Grass was also reduced, occupying secondary dominance in the sward with dead matter being most dominant from mid spring (17th November 2009) onwards (Figures 4.2 and 4.3). Largest observed standing biomass of both AACs (5%; 111 kg DM/ha) and grass (46%; 951 kg DM/ha) was in mid spring (November 17th 2009), which was earlier in the growing season than the previous year. The magnitude of reduction in mean grass component from the first to second year within the sward was minor compared to the major reduction observed for AACs (Table 4.3, Figures 4.2 and 4.3).
Table 4.4  Significance of main effects and interactions on the botanical cover (% VE cover) and biomass (% and kg DM/ha) dynamics following ANOVA of pasture data from a summer dry pasture community at the mid-altitude (680–700 m a.s.l.), north-facing (sunny) high country site at Broadspur hill block, Mt Grand Station, Central Otago, New Zealand, over the period October 2008-May 2010.

<table>
<thead>
<tr>
<th>Main pasture components</th>
<th>Adventive annual clovers</th>
<th>Grass</th>
<th>Dead matter</th>
<th>Total pasture mass</th>
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<tr>
<td></td>
<td>VE cover (%)</td>
<td>Biomass (kgDM/ha)</td>
<td>VE cover (%)</td>
<td>Biomass (kgDM/ha)</td>
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<tr>
<td>Standing (outside cage)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Year 1 GM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SP</td>
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<td>GM x SP</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Time of Harvest</td>
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<td>***</td>
<td>***</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>GM x SP x harvest</td>
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<tr>
<td>Time of Harvest</td>
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<td>***</td>
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<tr>
<td>GM x harvest</td>
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</tr>
<tr>
<td>GM x SP x harvest</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Accumulated (inside cage)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Year 1 GM</td>
<td>na</td>
<td>-</td>
<td>Na</td>
<td>**</td>
</tr>
<tr>
<td>SP</td>
<td>na</td>
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<tr>
<td>GM x SP</td>
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<td>na</td>
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<tr>
<td>Time of Harvest</td>
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<td>***</td>
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<td>SP x harvest</td>
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</tr>
<tr>
<td>GM x SP x harvest</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

NB  **VE cover**: visual estimate of cover (%), **GM**: grazing management in spring (continuous, early closure, late closure), **SP**: superphosphate application (Low–75 kg/ha and High–200 kg/ha of 30% Sulphur super), **Time of harvest**: Year 1–spring (Nov 11th, Dec 2nd), summer (Jan 27th), autumn (May 6th). Year 2–spring (Nov 17th), summer (March 1st), autumn (May 26th). Probabilities included when F values $P < 0.1; ***, ** and * indicate the treatment effect being significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ respectively, (–) not significant, na = no measurement made.
4.3.2.1 Adventive annual clovers

Adventive annual clover species (AACs) standing biomass increased during springtime of the first year from a starting point of 37% (565 kg DM/ha) on October 16th 2008 (early spring) reaching an observed maximum biomass of 758 kg DM/ha (35%) in late spring/early summer, 6.5 weeks later on December 2nd 2008. By mid-summer, 8 weeks later on January 27th 2009, AACs had declined to 20% (686 kg DM/ha). Fourteen weeks later in late autumn on May 6th 2009, the lowest AACs standing biomass (5%; 111 kg DM/ha) was observed (Figures 4.2 and 4.3). This comparatively small amount of AACs standing biomass was also observed the following spring on 17th November 2009, and was the highest observed level of standing biomass for AACs in the second year (Figures 4.2 and 4.3).

Figure 4.4 Standing biomass (kg DM/ha) of all adventive annual clover species (striated clover *Trifolium striatum*, suckling clover *T. dubium*, cluster clover *T. glomeratum*, haresfoot clover *T. arvense*) and sown clover species (white clover *T. repens* and subterranean clover *T. subterraneum*) over a two year period (October 2008–May 2010) at field site (680–700 m a.s.l.) in Broadspur hill block, Mt Grand Station, Central Otago, New Zealand. Error bars are ± SEM.

The pattern of standing biomass increasing to a maximum in late spring/early summer in the first year was not the same for all of the AAC species (Figure 4.4). *Trifolium striatum* and *T. dubium* did show this pattern, reaching maximum observed standing biomass of 352 and 178 kg DM/ha respectively in late spring/early summer (December 2nd 2008), (Figure 4.4, Plate 4.2). However,
T. glomeratum and T. arvense reached maximum observed biomass later in the growing season (Fig. 4.4). Peak standing biomass of T. glomeratum and T. arvense was 246 and 31 kg DM/ha in mid-summer (January 27\textsuperscript{th} 2009), 8 weeks longer into the growing season than T. striatum and T. dubium (Figure 4.4).

Plate 4.2 Close up view of the pasture sward in late spring/early summer showing Trifolium striatum in early flower (pink-white florets) and Trifolium dubium in full flower (yellow inflorescence with multiple florets), December 2\textsuperscript{nd} 2008 at Broadspur hill block at ~700 m a.s.l., Mt Grand Station, Central Otago, New Zealand.

In the second year, T. striatum had the most standing biomass of any clover species, with 105 kg DM/ha observed in mid spring (17\textsuperscript{th} November 2009), which represented 95% of the total clover biomass at that point in the growing season. All other adventive and sown species contributed <5% to the collective mean clover biomass in the second year (Figure 4.4).
To further illustrate the difference in clover abundance between the two years, standing biomass of the most dominant clover *T. striatum* in mid spring 2008 was 363 kg DM/ha. In mid spring 2009, standing biomass of this species was 105 kg DM/ha, 70% less than at the same point in the growing season 12 months previously (Figure 4.4).

**4.3.3 Growth (biomass/dry matter production)**

The growth pattern of adventive annual clover species (collectively) was different to that of sown clover species and grasses, with AACs growing more than sown clover species and grasses during spring and early summer of the first year.

Adventive annual clovers produced 45% and 56% of the total pasture growth over the periods 16th October–November 11th, and 11th November–December 2nd 2008 respectively (Table 4.5). Specifically, over 26 days in spring from 16th October–November 11th, AACs produced 169 kg DM/ha, at a growth rate of 6.5 kg DM/ha/day, while *T. repens*, *T. subterraneum* and grass produced 10, 25 and 74 kg DM/ha (3, 8 and 24% total pasture growth) respectively, at growth rates of 0.38, 0.96 and 2.8 kg DM/ha/day respectively (Table 4.5). Over the subsequent 21 days from mid spring to late spring/early summer (11th November–December 2nd) AACs produced maximum biomass of 561 kg DM/ha, at a higher growth rate of 28 kg DM/ha/day.

*Trifolium repens*, *T. subterraneum* and grass produced 27, 1, and 441 kg DM/ha (3, 0.1, 45% total pasture growth) respectively, at growth rates of 1.3, 0.05 and 21 kg DM/ha/day respectively (Table 4.5). Over the late spring–mid summer period from 2nd December 2008–January 27th 2009, grass growth overtook that of AACs, producing 767 kg DM/ha (49% total pasture growth) at a rate of 13.7 kg DM/ha/day, compared to 352 kg DM/ha for AACs (22% total pasture growth) whose growth had slowed down to 6.3 kg DM/ha/day (Table 4.5), reflecting the annual nature of these plants. Very little pasture growth occurred over the mid-summer–late autumn period from 27th January–May 6th 2009.

In the second year, very little AACs biomass (DM) production was observed in comparison to the relatively high first year production. This is reflected in the contrast in standing biomass between the two years (Figures 4.2 and 4.3). Grass growth was greater in comparison to the similar period in the previous year. Over 6 months, encompassing winter and early–mid spring of 2009, grass
had grown 1636 kg DM/ha (70% total pasture growth), at a growth rate of 8.3 kg DM/ha/day. In contrast, and unlike at the similar time the year before, AACs had only produced 43 kg DM/ha (2% total pasture growth), at a growth rate of 0.22 kg DM/ha/day (Table 4.5).

The growth pattern of individual AAC species was not the same, with *T. striatum* and *T. dubium* showing maximum growth earlier in the growing season than *T. glomeratum* and *T. arvense*. The latter two species continued to produce biomass further into summer (Table 4.5). Specifically, maximum growth by *T. striatum* (310 kg DM/ha) and *T. dubium* (138 kg DM/a) occurred over the mid spring–late spring/early summer period from 11th November–December 2nd 2008. Maximum growth by *T. glomeratum* (306 kg DM/ha) and *T. arvense* (46 kg DM/ha) occurred over the late spring/early summer–mid summer period from 2nd December 2009–January 27th 2009 (Table 4.5).

This growth pattern was also observed in the second year, despite AACs biomass production being vastly less. Growth by *T. striatum* (41 kg DM/ha) and *T. dubium* (2.4 kg DM/ha) was only observed over the period from late autumn–mid spring, while growth by *T. glomeratum* (3.5 kg DM/ha) and *T. arvense* (1.2 kg DM/ha) was only observed over the mid spring–late summer period in the second year (Table 4.5).
Table 4.5 Growth (dry matter production, kg DM/ha) of adventive annual clover species (striated clover *Trifolium striatum*, suckling clover *T. dubium*, cluster clover *T. glomeratum*, haresfoot clover *T. arvense*) and sown clover species (white clover *T. repens* and subterranean clover *T. subterraneum*), grasses, and total pasture over the period from October 2008–May 2010 at the mid altitude (680–700 m a.s.l.) summer dry high country field site, Broadspur hill block, Mt Grand Station, Central Otago, New Zealand.

<table>
<thead>
<tr>
<th>Year</th>
<th>Adventive annual clovers</th>
<th>White clover</th>
<th>Subterranean clover</th>
<th>Grasses</th>
<th>Total pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Year 1</td>
<td>Nov 11&lt;sup&gt;th&lt;/sup&gt; 2008</td>
<td>169</td>
<td>10</td>
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<td></td>
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<td>12</td>
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<td>0</td>
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<td>43</td>
<td>21</td>
<td>2</td>
<td>1636</td>
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<tr>
<td></td>
<td>March 10&lt;sup&gt;th&lt;/sup&gt; 2010</td>
<td>5</td>
<td>0</td>
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<tr>
<td></td>
<td>May 26&lt;sup&gt;th&lt;/sup&gt; 2010</td>
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<th>Subterranean clover</th>
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4.3.4 Influence of grazing management

Overall, the grazing management treatments imposed did not significantly influence the abundance (% visual cover or biomass) of AACs in either year (Table 4.4). However, two significant harvest times by grazing management interactions ($P<0.05$) were observed in the first year (Table 4.4).

On November 11th 2008 (mid spring), standing biomass harvested from continuously grazed (CG) plots (no netting; see Plate 4.1) contained significantly less AACs (466 kg DM/ha) than late closure (932 kg DM/ha) grazing management plots (Figure 4.5). Adventive annual clover mean standing biomass within CG plots (with netting) however, was not significantly greater than CG plots (no netting), but not significantly less than early closure (EC) or late closure (LC) plots (Figure 4.5). It is worth noting that LC plots had yet to be closed to grazing at the time of this first harvest and had been open to continuous grazing by Merino ewes and lambs since plots had been established on 16th October 2008.

On December 2nd 2008 (late spring/early summer) standing biomass harvested from EC plots contained significantly more ($P<0.05$) AACs (1482 kg DM/ha) than all other plots which had been continuously grazed up until December 2nd 2008; CG (no netting), CG (with netting) and LC plots contained 797, 805, and 674 kg DM/ha respectively (Figure 4.5).
Figure 4.5 Mean adventive annual clover (a) standing and (b) accumulated biomass (kg DM/ha), at harvest dates during the study period October 2008–May 2010, from Broadspur field site, 680–700 m a.s.l., Mt Grand Station, Central Otago, New Zealand. Error bars are ± SEM. Bars above data points indicate significant difference between plot means (LSD 5%).
The mean standing biomass of grasses inside grazing exclosure cages, harvested on January 27th 2009 (mid-summer) was significantly greater ($P<0.01$) from CG plots (2288 kg DM/ha) than in all other plots, with grass biomass from LC, EC and CG (netting) plots having 1381, 1500, and 1623 kg DM/ha respectively (Figure 4.6).

Overall, CG plots contained significantly more ($P<0.01$, Table 4.6) grass biomass than other plots, with 42% compared to 33.3, 32.8 and 20.9% within CG (netting), EC and LC plots respectively (Table 4.6).
Table 4.6 Mean accumulated biomass (% and kg DM/ha) of botanical components significantly influenced by spring grazing management and superphosphate fertiliser application at the mid-altitude, summer dry high country pasture site at Broadspur hill block, Mt Grand, Central Otago, New Zealand, over a two year period from October 2008–May 2010. ***, ** and * indicate the treatment effect being significant at \( P \leq 0.001 \), \( P \leq 0.01 \) and \( P \leq 0.05 \) respectively, (–) not significant, na = no measurement made, or not applicable. Different letters denote means that are significantly different @ 5% level.

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<tr>
<th>Botanical component</th>
<th>Significant treatment effect</th>
<th>Biomass (%)</th>
<th>Biomass (kg DM/ha)</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>Year 2</td>
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<td></td>
<td>Significance</td>
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Figure 4.7 Mean accumulated total pasture biomass (kg DM/ha), in relation to grazing management plots at harvest dates during the study period October 2008–June 2010, from Broadspur field site, 680–700 m a.s.l., Mt Grand Station, Central Otago, New Zealand. Error bars are ± SEM. Bars above data points indicate significant difference between plots (LSD 5 %).

Total pasture standing biomass harvested from within grazing exclusion cages of CG plots was the lowest and significantly less ($P<0.01$, Table 4.4) than all other grazing treatment plots in mid spring of 2008 (Figure 4.7).

Contrastingly, 12 months later, total pasture standing biomass from inside grazing exclosure cages was significantly greater ($P<0.01$) from CG plots (5780 kg DM/ha) than CG plots with netting (3820 kg DM/ha) on November 17th 2009 (mid spring), (Table 4.4, Figure 4.7). However, mean total pasture from EC plots (5003 kg DM/ha) and LC plots (4858 kg DM/ha) were not significantly less than CG plots, and not significantly more than CG plots with netting (Figure 4.7).
4.3.5 Influence of superphosphate fertiliser application

Overall, the application of superphosphate fertiliser did not have a significant influence on AACs biomass. However, a significant grazing management by superphosphate fertiliser interaction was observed in mid spring of the second year (Table 4.4). Adventive annual clover biomass was significantly greater \((P<0.05)\) within high (200 kg/ha) SP subplots of ES grazing management treatment plots. Specifically, AACs mean standing biomass was 84 kg DM/ha within high SP subplots compared to 27 kg DM/ha within low SP subplots (Table 4.7).

Table 4.7 Significant grazing management by superphosphate fertiliser interaction effect on mean adventive annual clover standing biomass (kg DM/ha) in the second year at the mid altitude (680–700 m a.s.l.) high country site, Brodspur, Mt Grand Station, Central Otago, New Zealand. LSD (5%)*: for comparison of means within the same grazing management treatment.

<table>
<thead>
<tr>
<th>SP fertiliser (30% Sulphur super)</th>
<th>Low (75 kg/ha)</th>
<th>High (200 kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous grazing</td>
<td>41.2</td>
<td>25.5</td>
</tr>
<tr>
<td>Continuous grazing (netting)</td>
<td>59.8</td>
<td>38.1</td>
</tr>
<tr>
<td>Early closure</td>
<td>26.8</td>
<td>83.7</td>
</tr>
<tr>
<td>Late closure</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>77.41</td>
<td></td>
</tr>
<tr>
<td>LSD (5%)*</td>
<td>40.41</td>
<td></td>
</tr>
</tbody>
</table>

The application of superphosphate fertiliser did appear to have a significant overall influence on the amount of mean dead standing biomass accumulated within grazing exclusion cages during the first year (Table 4.4). More dead matter was observed within low SP subplots than high SP subplots (Table 4.6).

4.3.6 Population dynamics of adventive annual clovers

4.3.6.1 Seed production

The mean number of seeds within an individual seedhead of each of the four resident adventive annual clover species was significantly different from each other \((P<0.001, \text{Table } 4.8)\). The clover species with the greatest seed number within a seedhead was in the order of \(T. \text{ arvense} > T. \text{ glomeratum} > T. \text{ striatum} > T. \text{ dubium}\), with 64, 24.9, 17.8 and 9.9 seeds per seedhead
respectively (Table 4.8). Grazing management influenced the mean seed number per seedhead, with early closure plots having significantly more ($P<0.05$) seeds per seedhead (Table 4.8).

Mean clover seed number within herbage harvested from a $0.2\text{m}^2$ quadrat was not significantly influenced by grazing management or superphosphate application, though some trends were evident (Table 4.8). Specifically, continuously grazed plots (with and without netting) had very similar mean clover seed numbers (125 and 126), while early closure and late closure plots had greater mean number of clover seeds at 144 and 156 respectively. More clover seeds were evident in low SP subplots (157) than high SP subplots (120), (Table 4.8).

There was a significant difference in the seed number of different clover species ($P<0.001$, Table 4.8). Specifically, mean seed numbers of *T. arvense* (8), *T. dubium* (62), *T. subterraneum* (0.4) and *T. repens* (2) within harvested herbage in mid-summer were not significantly different from each other. *Trifolium glomeratum* (491) and *T. striatum* (266) were different from each other, and different from the four other previously mentioned species ($P<0.001$, Table 4.8).
Table 4.8  Seed production by the six clover species at the mid altitude (680–700 m a.s.l.) pasture site in relation to spring grazing management (continuous, early closure, late closure), and superphosphate fertiliser application (Low-75 kg/ha and High-200 kg/ha of 30% Sulphur super) in January 2009 at Broadspur hill block, Mt Grand, Central Otago, New Zealand. ***, ** and * indicate the treatment effect being significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ respectively, (−) not significant, na = no measurement made, or not applicable. Different letters denote means that are significantly different @ 5% level.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean seed number (5 seedheads/species)</th>
<th>SEM</th>
<th>Seed number (within 0.2 m$^2$ quadrat)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T.$ <em>glomeratum</em></td>
<td>24.9 c</td>
<td>2.4</td>
<td>491 d</td>
<td>248.9</td>
</tr>
<tr>
<td>$T.$ <em>arvense</em></td>
<td>64 d</td>
<td>3.7</td>
<td>8 a</td>
<td>5.7</td>
</tr>
<tr>
<td>$T.$ <em>striatum</em></td>
<td>17.8 b</td>
<td>2.5</td>
<td>266 b</td>
<td>113.1</td>
</tr>
<tr>
<td>$T.$ <em>dubium</em></td>
<td>9.9 a</td>
<td>1.2</td>
<td>62 a</td>
<td>55.5</td>
</tr>
<tr>
<td>$T.$ <em>subterraneum</em></td>
<td>na</td>
<td>na</td>
<td>0.4 a</td>
<td>0.3</td>
</tr>
<tr>
<td>$T.$ <em>repens</em></td>
<td>na</td>
<td>na</td>
<td>2 a</td>
<td>1.7</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>4.1</td>
<td></td>
<td>130.6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spring Grazing Management</th>
<th>Mean seed number (5 seedheads/species)</th>
<th>SEM</th>
<th>Seed number (within 0.2 m$^2$ quadrat)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>25.6 a</td>
<td>2</td>
<td>125</td>
<td>74.8</td>
</tr>
<tr>
<td>Continuous (netting)</td>
<td>27.3 a</td>
<td>1.8</td>
<td>126</td>
<td>79.6</td>
</tr>
<tr>
<td>Early closure</td>
<td>34.1 b</td>
<td>4.2</td>
<td>144</td>
<td>63.7</td>
</tr>
<tr>
<td>Late closure</td>
<td>29.7 a</td>
<td>1.9</td>
<td>156</td>
<td>65.5</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>4.1</td>
<td></td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Superphosphate application</th>
<th>Mean seed number (5 seedheads/species)</th>
<th>SEM</th>
<th>Seed number (within 0.2 m$^2$ quadrat)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (75 kg/ha)</td>
<td>na</td>
<td>na</td>
<td>157</td>
<td>85.6</td>
</tr>
<tr>
<td>High (200 kg/ha)</td>
<td>na</td>
<td>na</td>
<td>120</td>
<td>56.1</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>na</td>
<td></td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance of effects</th>
<th>Species</th>
<th>Grazing Management</th>
<th>SP fertiliser</th>
<th>Species x GM</th>
<th>Species x SP fertiliser</th>
<th>GM x SP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>***</td>
<td>*</td>
<td>na</td>
<td>***</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

4.3.6.2  Seedling recruitment

Overall, mean seedling recruitment was greater in the second than first year with 24.4 and 9.7 seedlings/core respectively ($P < 0.001$, Table 4.9). Significant differences in seedling recruitment between clover species were evident, in both years ($P < 0.001$). In the first year, seedling
recruitment by _T. glomeratum_, _T. arvense_, _T. subterraneum_ and _T. repens_ was not significantly different at 4.7, 0.2, 0.4 and 0.8 seedlings per core respectively. _Trifolium striatum_ and _T. dubium_ seedling recruitment was the highest at 32.2 and 20.2 respectively, and significantly different from each other and all other species (_P_<0.001, Table 4.9). Seedling recruitment was greater in the second year for all adventive annual clover species but not sown clover species. _Trifolium arvense_ increased from 0.2 to 1.8 seedlings per core, but was still not significantly greater than _T. subterraneum_ and _T. repens_ at 0.6 and 0.7 seedlings per core respectively. _Trifolium glomeratum_ seedling recruitment had increased by more than three times to be the clover species with the second highest seedling recruitment at 15.8, while _T. striatum_ and _T. dubium_ seedling recruitment were equally the highest at 63.2 and 64.2 respectively. _Trifolium striatum_ and _T. dubium_ seedling recruitment in the second year were significantly greater (_P_<0.001) than _T. glomeratum_, but not significantly different from each other (Table 4.9).

Grazing management in spring produced no detectable influence on subsequent autumn seedling recruitment by any clover species, in either year (Table 4.9). However, there was a significant influence of SP fertiliser on seedling recruitment in the second year (_P_<0.001), and a significant fertiliser by clover species interaction in the second year (_P_<0.001), (Figure 4.8 and Table 4.9). Greater seedling recruitment was recorded from soil cores sampled from low SP subplots. Specifically, mean clover seedling number (mean of all clover species) was significantly greater (_P_<0.001) in cores sampled from low SP subplots (32.9) than from high SP subplots (15.9), (Table 4.9).

Figure 4.8 shows the influence of low (75 kg SP/ha) and high (200 kg SP/ha) SP fertiliser on seedling recruitment by the adventive annual clover species and sown clover species in autumn 2009 and autumn 2010. Three of the adventive annual clover species (_T. dubium, T. glomeratum_ and _T. striatum_) showed significantly greater (_P_<0.001) seedling recruitment under low SP fertiliser application in the second autumn. Specifically, within low SP fertiliser subplots, the mean seedling numbers of _T. dubium, T. glomeratum_ and _T. striatum_ were 85, 22 and 83 respectively, compared to 37, 10 and 44 seedlings respectively within high SP fertiliser subplots (Figure 4.8).
Table 4.9 Seedling recruitment of the six clover species in relation to spring grazing management (continuous, early closure, late closure), and superphosphate fertiliser application (Low-75 kg/ha and High-200 kg/ha of 30% Sulphur super) over a two year period at the mid altitude site (680–700 m a.s.l.), Broadspur hill block, Mt Grand, Central Otago, New Zealand. *** indicates an effect being significant at $P \leq 0.001$, (–) not significant, na = no measurement made, or not applicable. Different letters denote means that are significantly different @ 5% level.

<table>
<thead>
<tr>
<th>Species</th>
<th>Clover seedling recruitment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 1</td>
<td>SEM</td>
<td>Year 2</td>
</tr>
<tr>
<td>T. glomeratum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. arvense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. striatum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. dubium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. subterraneum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. repens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>7.5</td>
<td>12.03</td>
<td></td>
</tr>
</tbody>
</table>

Spring Grazing Management
- Continuous: 11  4.2  24.2  7.7
- Continuous (netting): 9.2  3.5  23.6  9.3
- Early closure: 11.6  6.4  24.7  9.6
- Late closure: 7.1  1.8  25.1  10.2
- LSD (5%): na  na

Superphosphate application
- Low (75 kg/ha): 10.7  4.4  32.9 b  9.2
- High (200 kg/ha): 8.8  3.6  15.9 a  9.2
- LSD (5%): na  6.95

Grand Mean: 9.7  1.23  24.4  1.23

Significance
- Species: *** ***
- Species x GM: – –
- Species x SP fertiliser: – ***
- Species x Year: *** ***
- Species x SP x Year: *** ***
- Grazing Management: – –
- SP fertiliser: – ***
- SP fertiliser x Year: *** ***
- Year: na ***
Figure 4.8 Influence of low (75 kg/ha) and high (200 kg/ha) superphosphate fertiliser application (30% Sulphur super) on seedling recruitment of the six resident clover species over a two year period (2008–2010) at a summer dry, upland pasture community at the mid-altitude (680–700 m a.s.l.), north-facing (sunny) high country site at Broadspur hill block, Mt Grand Station, Central Otago, New Zealand. Error bars are ± SEM.
Table 4.10 Mean standing (outside grazing exclusion cages) and accumulated (inside grazing exclusion cages) pasture biomass over a two year period at Broadspur hill block, Mt Grand, Central Otago, New Zealand. ***, ** and * indicate the difference between standing and accumulated biomass as being significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ respectively, (−) not significant, na = no measurement made. NB VE cover = visual estimate of botanical cover.

<table>
<thead>
<tr>
<th>Time of harvest</th>
<th>Adventive annual clover</th>
<th>Total grasses</th>
<th>Dead</th>
<th>Total pasture mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VE cover (%)</td>
<td>Biomass (%)</td>
<td>Biomass (kg DM/ha)</td>
<td>VE cover (%)</td>
</tr>
<tr>
<td>Year 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November 11th</td>
<td>na</td>
<td>–</td>
<td>–</td>
<td>na</td>
</tr>
<tr>
<td>December 2nd</td>
<td>na</td>
<td>–</td>
<td>**</td>
<td>na</td>
</tr>
<tr>
<td>January 27th</td>
<td>na</td>
<td>–</td>
<td>*</td>
<td>na</td>
</tr>
<tr>
<td>May 6th</td>
<td>na</td>
<td>–</td>
<td>–</td>
<td>na</td>
</tr>
<tr>
<td>Year 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November 17th</td>
<td>**</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>March 1st</td>
<td>–</td>
<td>*</td>
<td>*</td>
<td>–</td>
</tr>
<tr>
<td>May 26th</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
4.4 Discussion

4.4.1 Year to year variation

4.4.1.1 Adventive annual clover species

The large variation in mean adventive annual clover species abundance between the two years at this north-facing, mid altitude high country pasture site can be attributed to the contrast in rainfall quantity during spring and early summer between 2008 and 2009 (Figure 4.1). Adventive clover species biomass (% DM and kg DM/ha) was ~13.7 times greater in the first year (2008–2009 season) than the second year (2009–2010 season) of sampling (Table 4.3, Figures 4.2, 4.3, and 4.4). Rainfall during the growing season of 2008 in September, October, November and December was much greater at 75, 37, 42 and 146 mm respectively than that of the following year (2009) at 10, 29, 4 and 34 mm respectively. This was particularly evident in September and November of 2009 when rainfall was considerably lower than the 60 year mean for those spring months (Figure 4.1). September and November rainfall in 2009 was only 13% and 9.5% of the year before. The 4 mm recorded for November rainfall in 2009 at the field site was 92% less than mean rainfall (60 year average) for November (Figure 4.1). Rainfall recorded at Mt Grand in general in November 2009 was the lowest on record since 1937 (E. Gibson, Mt Grand Station farm manager, pers. comm.).

This substantial difference in spring and early summer rainfall between the two years (specifically low rainfall in September and November of 2009, Figure 4.1) was therefore the primary factor causing low soil moisture levels during the growing season of the second year. As a result, soil moisture availability was low for all actively growing pasture plants in general, especially young annual clover plants and any late (winter)-germinated seedlings. The low rainfall in September of the second year constrained growth and development of young AACs plants and winter/early spring germinated AACs seedlings leading into a very dry November. October rainfall variation between 2008 and 2009 was not as large (37 mm compared to 29 mm respectively) as observed for September and November, with October rainfall in 2009 being 78% of that in 2008. However, this was 22% less rainfall than the same time the year before, which occurred during the early–middle part of the growing season and would have done little to elevate the low existing soil moisture conditions already evident from low September rainfall and the subsequently high soil moisture deficits during the exceptionally dry November of 2009.
The soil cover of hill and high country areas at Mt Grand is shallow and stony, with low water storage capacity (Duncan et al. 1997) making localised soil moisture levels very dependent on the frequency of rainfall (re-wetting) events rather than the total rainfall amount in spring–summer (Bircham & Gillingham 1986). North-facing hillsides at Mt Grand dry out quickly, within one-two days, after a rainfall event (T.M.R. Maxwell, personal observations during data collection at Mt Grand; E. Gibson pers. comm.). Though the frequency of monthly rainfall events during the growing season at the Mt Grand field site was not measured, the recorded total rainfall for each month gives a primary indication of soil moisture levels at the site during the spring and early summer.

Hepp et al. (2003) attributed a 2.5-fold difference in clover abundance (Trifolium repens, T. subterraneum, T. dubium and T. glomeratum) to higher soil moisture availability and more even rainfall distribution in the second season in comparison to the first season of sampling in summer dry North Island east coast hill country. Though available water capacity (AWC %), as a measure of soil moisture levels, was not measured at this South Island summer dry high country field site, unlike Hepp et al. (2003) and thus cannot be quantified, we can assume spring and early summer soil moisture availability for dominant sward components of AACs and grasses was greater in the first year, allowing for the larger standing biomass production by the adventive clover component that was observed when compared to standing biomass at the same period in the second year. In essence, the onset and magnitude of the periodic summer drought was delayed at this typical north-facing pasture site in the first year. In contrast, and perhaps reflecting the more usual annual rainfall levels, high soil moisture deficits, characterising the late spring–summer drought, would have been evident much earlier in the growing season of the second year. Specifically, in November, this would limit the pasture production (AACs and grass biomass levels), as was observed (Table 4.3, Figures 4.2, 4.3 and 4.4).

The presence of the various grass species (perennial and annual) would have contributed to the low level of observed AACs in the second year due to competition for already scarce soil moisture. Perennial grasses and volunteer annual grasses dry out the soil surface layer to the detriment of T. subterraneum seedling establishment and growth (Dear & Cocks 1997). The suite of perennial grass species present at the site, such as drought-tolerant Rytidosperma spp., Agrostis capillaris L., Austrostipa nodosa, Dactylis glomerata L., and others such as Anoxanthum ordoratum L., Poa pratensis L., Lolium perenne L., Elymus spp., and Arrhenatherum elatius L., in conjunction with several volunteer/adventive annual grass species such as Bromus diandrus Roth and Bromus tectorum L., would have been actively
utilising soil water to the detriment of young AACs plant growth and hence biomass accumulation.

4.4.1.2 Annual pasture biomass, dead matter and grasses

Despite the large variation in clover content, mean annual standing biomass of pasture was similar (2433 and 2500 kg DM/ha) between the two years (Table 4.3). These values are comparable to other South Island hill country (200–600 m a.s.l.) annual pasture biomass values recorded in low rainfall/summer dry areas (350–760 mm/year) ranging from 2800, 2100, and 1000–2000 kg DM/ha (White et al. 1972; Radcliffe & Cossens 1974; Vartha et al. 1982 respectively) as well as summer dry (800 mm/year) North Island hill country annual pasture biomass (2322 kg DM/ha) (Gillingham et al. 1998).

Dead material was more prevalent in the sward, while grasses were slightly less abundant in the second year (Table 4.3). Greater mean dead matter levels and less grass dominance in the second year can also be attributed to low spring and early summer rainfall in 2009, as moisture-stressed pasture plants mature quicker, or die, and generate litter and standing dead matter earlier in the sward. Additionally, remnant attached dead matter (grass stems and seedheads) from the previous moist years’ abundant growth would have contributed to the mean dead matter level in the second year, due in part to relatively lax grazing pressure during the spring and early summer flush. This relative surplus in pasture could not be fully utilised with greater grazing pressure from an increased stocking rate.

Dead matter accumulation is related to lower digestibility values (~40%, Vartha et al. 1982). The dynamics of pasture growth and decay play a large role in the quality of standing herbage in hill and high country (Clarke 1977).

4.4.1.3 Seedling recruitment

Seedling recruitment by the three predominant AAC species (Table 4.9 and Figure 4.8) was greater in the second autumn than the first. The primary reason for this would have been that a greater litter layer (unattached dead matter) was present in the first autumn. As a consequence the moist spring and early summer season and associated lax grazing pressure, during which abundant clover and grass accumulation occurred, resulted in a large unattached dead matter content (litter layer) in autumn (Figures 4.2 and 4.3). This occurred during the time of clover seed germination and seedling emergence in autumn. Litter layers inhibit seedling recruitment of grassland species (Jensen & Gutekunst 2003).
Following a very dry mid-spring and more usual summer dry period in the second year, considerable grass plant death would have occurred leaving colonising space for annual clovers and/or less competition from the remaining live grass component (Edwards & Crawley 1999) during autumn.

Another possible reason for the greater adventive clover seedling recruitment observed in the second year is a larger proportion of grass seed germinated in the first autumn owing to mass viable seed production during summer following the moist spring and early summer conditions. This would have resulted in fewer available grass seeds to germinate from the soil surface seed bank in the second autumn, again allowing more clover seedlings to occupy soil space and establish. Little carry-over of grass species from season to season in comparison to annual clovers was observed in Mediterranean grassland in northern Syria of ~350 mm annual rainfall (Russi et al. 1992).

Hard-seededness (seed coat impermeability) is one strategy of annual legumes to ensure persistence under conditions of low or zero seed set (Reed et al. 1989). The hard seed proportion of the adventive annual clover seed rain from the first year may have been permitted to germinate in the second autumn following the hard seed-softening conditions / breakdown of hard seed over the dry summer period from fluctuating (diurnal) soil surface temperatures (Quinlivan & Millington 1962; Lodge et al. 1990).

Greater fixed N inputs as a result of the moist spring and early summer-driven AACs flush during the first year (Boswell et al. 2003b) would have benefitted the grass component of the summer dry hill pasture community, made up of established perennial grass species and regenerative annual species (Table 4.3). Sward legume biomass and N-fixation inputs are strongly related (Ledgard et al. 1988; Lucas et al. 2010). This would have increased N inputs to the plant-available soil nutrient pool. This would in turn have benefitted the grasses component at the expense of AACs seedling population in autumn of the first year via stronger grass competition (established perennial plants and seedlings of annual and perennial species) in response to more soil N availability. The abundant summer grass growth and associated seed production in the first year (T.M.R. Maxwell, field observations) would have added to the grass competition with the regenerating AACs component in late winter-early spring of the second year. Dear et al. (1998) highlighted that reduced early growth of *T. subteraneum* seedlings in a mixed sward of *Phalaris aquatica* L., *Dactylis glomerata* L. and
Danthonia richardsonii was due to competition for ground space, soil water and nutrients from established plants of these perennial grass species.

4.4.2 Sown clover species content

Trifolium repens and T. subterraneum were not dominant clover species at any time over the two year period, adding further support to the contention that T. repens (Hoglund 1990; Knowles et al. 2003) and T. subterraneum (Power et al. 2006) struggle to persist and make a valuable contribution to pastures of north-facing hill slopes in the summer dry zone. Overall, these two sown species occupied less ground cover and contributed less biomass to the sward than the weed and herb species at this site (Table 4.2).

4.4.2.1 Trifolium repens

Periodic summer soil moisture stress compromises the success of T. repens (persistence at abundant levels) in northerly-facing summer dry hill and high country pasture leading to low T. repens content within these swards. Fundamentally, this is a reflection of this perennial species’ different phenology compared to the three predominant adventive annual species at this site (T. striatum, T. glomeratum, and T. dubium), making it poorly-adapted and thus less successful in a periodically moisture-stressed environment, characteristic of northerly-facing summer dry hill and high country areas (Valentine & Matthew 2000; Lonati et al. 2009).

All the AACs have earlier germination and emergence, and greater seedling growth at cooler temperatures then T. repens (Lonati et al. 2009). The greater ability of T. striatum, T. glomeratum and T. dubium to persist in larger proportions during dry years, and thrive during moist years results in sward legume dominance by these annual species over T. repens at this site, despite periodic oversowing (every 3–5 years) of the latter.

As a perennial clover species whose predominant mode of proliferation is stolon growth, development and branching during late spring and summer (Valentine & Matthew 2000) more so than seedling recruitment (Chapman 1987), its ability to increase biomass contribution to sward clover content at this north-facing mid altitude site is compromised as soil moisture deficits occur in most years from late spring/early summer onwards (Hoglund 1990; Knowles et al. 2003; Power et al. 2006) from which T. repens plant mortality is high and subsequent recovery is poor (Knowles et al. 2003).
Trifolium repens has colonised all suitable/favourable sites at Mt Grand and any further spreading of seed via broadcast oversowing need not continue as this is unlikely to influence its content within the pastures (Power 2006; R. Lucas pers. comm.). The introduction of earlier flowering *T. subterraneum* cultivars has been recommended, particularly onto drier, steeper hillsides dominated by *T. striatum* and *T. glomeratum* in the mid to lower altitude zone.

### 4.4.2.2 *Trifolium subterraneum*

The grazing management regime of north-facing hill blocks at Mt Grand (Materials and Methods section) in combination with very dry years from climatic variability has not favoured the level of seed production required for the adequate regeneration of *T. subterraneum* to be a successful contribution to the sward legume content. Little wide-spread oversowing of *T. subterraneum* has occurred since the 1960s in comparison to *T. repens* which has been oversown every 3–5 years. In addition, *T. subterraneum* has probably been preferentially grazed on sunny aspects through the years at Mt Grand, being a preferred clover species for sheep (Hyslop et al. 2003) to the detriment of its abundance in the sward (R. Lucas pers. comm.).

Ates et al. (2006) stated that in a drier than average spring season (low October rainfall), selective grazing of *T. subterraneum* may be detrimental to seed production resulting in reduced seedling numbers in the following autumn. In dryland Canterbury Plains pasture conditions, *T. subterraneum* seedling populations decreased in response to later grazing closure dates in spring; 3850, 2950, 2100 and 1700 seedlings/m² were recorded at 2, 4, 6 and 8 weeks from the point of the first visible flower respectively (Ates et al. 2008). Under the grazing regime at Mt Grand of continuous stocking at 4.6 SU/ha from mid-autumn (late April) through to mid-winter (mid-July) then spelling until mid-September whereupon grazing continues throughout spring and part of summer (until late February), there is continuous grazing pressure throughout the flowering and seed set period for *T. subterraneum* plants of the mid-late flowering variety Mt Barker.

Seasonal dry matter production is an important indicator of a plant’s adaptability to an environment. Annual legumes depend on seed production and seed reserves for regeneration (Lodge et al. 1993). Three of the adventive annual clover species (*T. striatum*, *T. glomeratum* and *T. dubium*) produced more DM (Table 4.3, Figures 4.2, 4.3 and 4.4) and more seeds (Table 4.8) than the sown clover species at this summer dry hill pasture site. *Trifolium*
striatum, T. glomeratum and T. dubium showed larger seedling populations in consecutive autumns (Table 4.9 and Figure 4.8) than T. arvense and the sown species at this site. It appears that the three predominant species have the ability to grow and produce viable seed in most years in this environment before the onset of late spring–summer soil moisture deficits, followed by autumn regeneration every year from the large reserve of seed in soil seed bank.

4.4.3 Adventive annual clover species content

All the adventive annual clover species present at this site have lower base temperatures for germination and lower thermal time requirements for seedling emergence and seedling growth than T. repens (Lonati et al. 2009) which has been the main clover species used in broadcast oversowing events over north-facing (sunny, exposed aspects) at Mt Grand in the last 30 years.

The ability to germinate and establish seedlings at lower temperatures in autumn means T. striatum, T. glomeratum and T. dubium are better adapted than T. repens at regenerating and producing biomass under the climatic conditions of north-facing hillsides on Mt Grand Station, allowing these species to persist more abundantly despite periodic (every 3–5 years) broadcast-oversowing of T. repens. As annual plants, these adventive clover species are able to complete their lifecycle (germinate, establish, flower and produce seed) before the onset of soil moisture deficits typical of sunnier northern aspects in late spring–summer (Lambert & Roberts 1976) that are detrimental to T. repens growth and stolon propagation in most years. Consequently, adventive annual clover species abundance during moist years (2008–2009 season), and persistence in dry years (2009–2010), is greater than T. repens at this north-facing site. These adventive annual clover species appear more ecological successful due to their regenerative ability (Lonati et al. 2009) and reproductive strategy (Norman et al. 2005) in this summer dry environment; ecological success is manifested in the persistence of these species, especially T. striatum from year to year.

Trifolium striatum along with Rytidosperma spp. (Danthonia grasses) were the most abundant components of unimproved vegetation at a 500 m a.s.l. hill side of north-westerly aspect in North Canterbury, where soil moisture stress was evident from late November to late February (Vartha at al. 1982). Observations obtained from this study at Mt Grand Station are in phase with these earlier observations of Vartha et al. (1982).
Trifolium striatum showed increased cover (%) through time on reclaimed land used previously for open-pit coal mining in a Mediterranean environment in Spain that had previously been hydro-seeded with perennial herbaceous species as the primary method of re-vegetating hill sides (Gonzalez-Alday et al. 2008). Summer drought resulted in the reduced vegetative cover of hydro-seeded perennial species such as Trifolium repens, Trifolium pratense L., Festuca spp., Lolium perenne L., Phleum pratense L., Lotus corniculatus L. and Medicago sativa L., allowing native species to colonise from surrounding vegetation, of which T. striatum and T. glomeratum were prominent (Alday et al. 2010).

Trifolium glomeratum is regarded as an annual species with mass seeding ability. Under water-stressed conditions, seed production is maximised by high reproductive allocation and small seed size (Smith et al. 1998). Similarly, Norman et al. (2005) described T. glomeratum as a small-seeded species with a generalist reproductive strategy, producing as many seeds as possible in each season, with little hard-seededness.

Trifolium dubium is a generalist species that persists in a wide range of environments (both moist and dry) and is the most common and widespread adventive clover species throughout New Zealand, growing in a range of soils (Boswell et al. 2003a). Trifolium dubium appeared better adapted to low soil fertility status relative to T. repens in moist (1280 mm) North Island hill country, with the capability to grow better where severe summer moisture stress (drought) can occur (Lambert et al. 1986). These authors reported that T. dubium showed greater winter and early spring growth than T. repens.

Maxwell et al. (2010) reported that neither shady, moderately sunny, or sunny aspects had any significant influence over T. dubium cover at Mt Grand in early summer, being equally abundant on drier, north-facing hill slopes (5%) and north-east/west facing slopes (5.4%), though slightly greater on moister, south-facing hill slopes (7.3%). Trifolium dubium appears to have a scrambling growth and regeneration strategy for a wide range of micro-climate sites that differ in soil moisture regime (Maxwell et al. 2010).

Trifolium arvense is predominant in undeveloped semi-arid environments in the Mackenzie Basin (Boswell et al. 2001) and a species that exists more abundantly in areas of light, shallow, sandy or stony soil, river beds, and within lower altitude modified tussock grasslands (Boswell et al. 2003a), shingle fans, and more exposed, sunny outcrops (T.M.R. Maxwell, field observations). Its relatively small presence at this site is a reflection of greater grass competition and lower bare ground cover from higher rainfall in most years in contrast to more semi-arid environments (~500 mm rainfall/year).
*Trifolium subterraneum* is an annual clover species that also germinates and establishes seedlings at lower temperatures than *T. repens* (Lonati et al. 2009) however, its overall sward presence was no greater than *T. repens* in either year. *Trifolium subterraneum* cv. Mt Barker has not been oversown at Mt Grand in the last four decades (R. Lucas pers. comm.). Periodic preferential grazing of *T. subterraneum* plants by merino sheep in combination with a grazing management regime that may negatively influence seed production has contributed to the observed low abundance of *T. subterraneum* at this site. Additionally, *T. subterraneum* cv. Mt Barker appears susceptible to disease (R. Lucas; D.J. Moot, pers. comm.).

### 4.4.4 Grazing management effect

Grazing pressure was evident during the experimental period as significant differences between accumulated and steady state biomass was observed (Table 4.10). Any significant grazing management treatment observed, such as greater AACs within early closure plots in late spring/early summer (December 2\textsuperscript{nd}) of the first year produced no detectable carry over effect into the subsequent autumn, such as greater clover seedling recruitment, or in the following spring growing season, such as greater clover standing biomass.

Ates et al. (2006) found that *T. glomeratum* cover was not influenced by low (10 ewes/ha) or high (20 ewes/ha) stocking rate, ranging from 15.3\textendash16.1\% in a typical summer dry lowland Canterbury plains environment of shallow, stony soil cover.

Reasons for this appear to be that the adventive annual clover seed bank is high or at maximum already, prior to the grazing management treatments being imposed. The grazing management treatments imposed had no significant influence on AACs reproduction. The adventive annual clover content appears high during a moist season at this site, with *T. striatum* > *T. glomeratum* > *T. dubium* > *T. arvense* in dominance. Three of these species are occupying/have reached a climatic niche that fluctuates in response to spring rainfall amount and distribution, while the current levels of grass competition, low to moderate soil P and S fertility, and in-preferential grazing pressure from the onset of flower maturation (T.M.R. Maxwell; Chapter 7) are not influencing the abundance of the adventive species, or are secondary in dominance to the primary overriding factor of soil moisture regime during late winter–late spring/early summer, being a function of spring and summer rainfall quantity and frequency.
It is difficult to extrapolate differences between grazing treatments due to the cross site variation in soil depth ranging from 5 to 30 cm plus (due to underlying schist rock), (T.M.R. Maxwell, field observations) and patchiness of botanical component; in situ differences between grazing management plots, such as elevated grass dominance in continuously grazed plots (Figure 4.6) and elevated AACs dominance in late closure grazing management plots (Figures 4.4 and 4.5) appearing to be naturally present and unrelated to the treatments imposed.

Furthermore, though grazing pressure was evident (Table 4.10), the moist conditions in the first year caused a large pasture standing biomass response that lasted into mid-summer. Grazing pressure at the hill block was perhaps not strong enough in the moist first year, relative to usual conditions, but was realistic of the management that occurs, reflecting the extensive system and climatic variation from year to year and thus the conservative stock number policy that needs to be exercised for risk aversion. Too few livestock carried is better than too many livestock carried in such an extensive hill and high country system where dry conditions and subsequent soil moisture stress can set in quickly (1–2 weeks) during late spring–summer (E. Gibson pers. comm.).

4.4.5 Fertiliser effect in second year

Overall there was little to no response by the AACs or grasses component to superphosphate fertiliser application. Reasons for this include a low nutrient requirement for optimum growth of the prevalent species (*T. striatum*, *T. glomeratum* and *T. dubium*); specifically a low S requirement for optimum DM production (Maxwell et al. 2012) and adaptation to low soil P levels (Dodd & Orr 1995).

Beale et al. (1993) reported that *T. striatum* and *T. arvense* as species restricted to light textured soils in Morocco, with the former species being associated with soils of very low P status. A recent glasshouse pot trial demonstrated relatively lower DM response of *T. striatum* to increasing P fertility in a South Island high country soil, compared to *T. repens* cv. Nomad, *T. subterraneum* cv. Mt Barker, *T. dubium*, *T. glomeratum* and *T. arvense* (T.M.R. Maxwell; Chapter 5). Furthermore, the duration of pasture measurements from this field experiment may not have been long enough to observe a significant SP response.
Only a small AACs response in high SP subplots of one grazing management treatment plot was observed during the second year, 12 months after fertiliser application. However, this only occurred within early closure grazing management plots, suggesting in situ variation once again rather than overall SP fertiliser influence.

Greater dead matter was observed within low SP subplots during the first year. More dead matter in low SP subplots can perhaps be attributed to greater decomposition rates in the high SP subplots due to enhanced/greater microbial activity breaking down litter material within the sward near the soil surface at faster rates than within low SP subplot areas. Lodge et al. (2006) reported fertilised (125 kg/ha single superphosphate; 11 kg P and 14 kg S/ha) and continuously grazed native pasture sites of lower altitude (510 m a.s.l.) north-west facing slopes of temperate northern New South Wales (694 mm/annum rainfall) showed higher litter DM losses and relative rates of decomposition compared with unfertilised and continuously grazed native pasture sites.

Overall, significantly more *T. striatum*, *T. dubium* and *T. glomeratum* clover seedlings were evident in low SP subplots, suggesting an affinity for lower S and P fertility by these species, especially *T. striatum* and *T. dubium*. These species may exhibit optimum germination, seedling emergence and development due to an adaptation to lower fertility soil conditions. Maxwell et al. (2010) (T.M.R. Maxwell; Chapter 3) found greater *T. striatum* cover (%) was associated with lower soil P levels in mid–lower altitude range at Mt Grand. However, no association was observed between the three predominant annual clover species and soil S fertility level. Maxwell et al. (2012) (T.M.R. Maxwell; Chapter 6) concluded that all four of these adventive annual clover species examined here require low S availability for optimum DM production, under conditions of optimum soil pH, P fertility, and micro-nutrient status. Under low to medium soil P fertility, all four adventive clover species were able to show optimum DM accumulation in a glasshouse trial (T.M.R. Maxwell; Chapter 5).

### 4.5 Conclusions

Pasture sampling occurred over two consecutive years in which the nature of the growing season and clover species dynamic was determined by distinctly different spring rainfall quantity from the first to second year.
Variation in seasonal rainfall distribution from year to year, specifically during the spring to early summer period (September–November and early December) was the primary determining factor for adventive annual clover species abundance in north-facing summer dry high country grassland, owing to its effect on soil moisture availability and thus pasture biomass production and associated seed set.

Spring grazing closure positively influenced the abundance of adventive annual clover species in a moist season. However, this produced no significant increase in seed production, and no detectable positive influence on seedling recruitment in following autumn, or adventive annual clover herbage mass in the following dry spring, was observed.

*Trifolium striatum* was the most dominant clover species, either in moist or dry spring–early summer conditions. *Trifolium glomeratum* and *T. dubium* were secondarily-dominant to *T. striatum* in a moist spring–early summer. *Trifolium arvense, T. repens* and *T. subterraneum* were the least abundant clover species in either a moist or dry season.

Spring grazing exclusion and superphosphate fertilizer application had no to little short-term impact on the abundance of naturalised adventive annual pasture legumes, persistent in grazed summer dry New Zealand South Island hill and high country.

Overall, no detectable response to superphosphate application was observed by adventive annual clovers that appear to be adapted to low P environments and require low levels of S to grow well. *Trifolium striatum* was the only adventive annual clover species that showed a small DM response to superphosphate fertiliser input, in the second year after fertilizer application.

Naturalised, resident adventive annual legumes exhibit regeneration and persistence strategies allowing them to annually re-establish and grow in dry hill and high country pastures; they are ecological successful in dry, extensively grazed hill and high country pastures.
Chapter 5
Phosphorus response and efficiency of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions

This chapter has been submitted to the New Zealand Journal of Agricultural Research, November 2012.

5.1 Introduction
Phosphorus (P) is second only to nitrogen (N) as the key macro nutrient driving the productivity of legume-based grazed pasture systems in New Zealand hill and high country. Often the sole nitrogen (N) inputs to these systems are sourced from sward legumes (clovers), through biological N fixation (Haynes & Williams 1993). This N input, as the key driver of overall pasture yield, is strongly influenced by soil P fertility (Moir et al. 2000). In general, higher soil P fertility often allows for a larger proportion of pasture legume to be present and to persist in the sward, resulting in greater annual biological soil N inputs (Gillingham et al. 2008). As such, highly productive legume species and pastures are generally adapted to high fertility soil conditions and do not perform well in infertile and/or acid soil conditions (Haynes & Williams 1993). ‘Improved’ pastures therefore require annual maintenance fertiliser inputs, such as single superphosphate (SSP) to elevate soil P and sulphur (S) fertility and sustain high production. In contrast, annual fertiliser inputs to these farming systems are, in reality, low, driven by the economics of fertiliser inputs. As a result, many hill and high country soils in New Zealand have soil fertility levels far below optimum for many common pasture legume species, such as white clover (Trifolium repens L.). As world P resources rapidly decline, fertiliser prices are likely to increase (Gilbert 2009; Vaccari 2009). Therefore the long-term sustainability of these agroecosystems may depend on greater P efficiency, involving sustained production with reduced fertiliser P inputs.

The sustainability of hill and high country pastoralism in New Zealand is also currently under considerable pressure, resulting from central government policy to retire significant areas of high altitude land (>1000 m a.s.l.) to improve indigenous grassland conservation and recreation resources. This has increased the need to improve pastoral primary productivity of
the remaining middle to lower altitude land (900–300 m a.s.l.) used for fine wool and meat (lamb and beef) production from extensive livestock grazing agroecosystems. An important component of pastoral intensification is to increase legume abundance, so as to provide increased feed and nitrogen inputs to nitrogen deficient, hill country grassland. However, the establishment and persistence of sown legume species such as white clover (Trifolium repens L.) and subterranean clover (Trifolium subterraneum L.) is often limited in summer dry, low soil fertility areas (Knowles et al. 2003; Power et al. 2006). This is in contrast to the common presence of other naturalised, adventive and unsown legumes, such as cluster clover (Trifolium glomeratum L.), haresfoot trefoil (T. arvense L.), striated clover (T. striatum L.) and suckling clover (T. dubium Sibth) (Power et al. 2006) that may be more suited to the low soil fertility and microclimates that exist on these hill slopes (Boswell et al. 2003; Maxwell et al. 2010).

Soil plant-available nitrogen (N) levels, sourced from pasture legumes, have been positively associated with long-term fertiliser phosphorus (P) and sulphur (S) use in NZ hill country (Moir et al. 1995; 1997). Recent N fertilizer trials (Gillingham et al. 2004; Smith et al. 2004) suggest feed production in lower altitude hill and high country is dependent on N (fertiliser) inputs. With the suite of naturalised adventive annual legumes that are adapted to the dry and semi-arid grasslands in New Zealand (Boswell et al. 2003), and hill and high country soils often being deficient in N (Haynes & Williams 1993), more knowledge, both ecological and agronomic regarding such legume species is needed. Information of this nature would benefit land managers and farmers of hill and high country areas towards improving productivity and sustainability of these grassland ecosystems. How these naturalised adventive legumes respond to added P to the plant-available soil P pool is an important question. Further, establishing a ‘critical’ level of available P; namely at which the efficiency of fertiliser use is at or close to 100% for these species in low fertility, extensive farming systems, and how much P should/can be added to increase plant-available P to the critical level for these species (Syers et al. 2010) is of subsequent great importance for fertiliser best management practices (FBMPs) towards sustainability and wider ecosystem and environmental health (Roberts 2010). By definition, annual legumes release N into the environment every year, via decomposition of plant matter and symbiotic N fixation. Such pulses of N into an N-deficient system via increased plant populations of these grazed legumes as a result of P (and/or S) additions to the ‘critical’ level maybe beneficial to such agroecosystems.
The soil of NZ hill and high country environments are traditionally low in plant-available P and S, (Moir et al. 1995). These two macronutrients are critical for pasture legume growth and development, especially as legumes have a greater requirement for P than grass species (Caradus 1980). Much research has been conducted investigating white clover (*Trifolium repens*) dry matter and growth responses to added P in New Zealand (Caradus & Snaydon 1986; Caradus et al. 1995). Much research has been conducted in Australia investigating subterranean clover (*T. subterraneum*) responses to added P (Paynter 1993; Bolland & Paynter 1994; Bolland 1995; Cayley & Hannah 1995; Cayley et al. 1998). The literature addressing responses of *T. subterraneum* to added P under NZ conditions is limited. Furthermore, little research has been conducted looking at the growth and dry matter production of naturalised adventive annual legumes (Brock 1973). Apart from *T. striatum* presence and abundance having been associated with low soil P levels (Beale et al. 1993; Maxwell et al. 2010), it is essentially unknown how these naturalised pasture legumes (cluster, haresfoot, suckling and striated clover) respond to key factors limiting growth in an extensively grazed hill/high country environment; namely, low or variable P fertility (Dodd & Orr 1995).

This experiment examined the vegetative dry matter response and phosphorus uptake of four (non-commercial) naturalised, adventive annual legume species (cluster, haresfoot, suckling and striated), one commercial annual legume species (subterranean clover) and one commercial perennial legume species (white clover) to added P in a New Zealand (central Canterbury) high country soil, grown under glasshouse conditions. The objective of this research was to determine the influence of P supply on the DM yield of these six pasture legume species (five annual and one perennial) grown in a typical Southern YBE soil under glasshouse conditions. In addition, P uptake, and P utilisation / response efficiency of the different pasture legume species was investigated.
5.2 Materials and Methods

5.2.1 Soil sampling

The soil used was a ‘Cass Series’ High Country Southern Brown soil (NZ classification: Upland Allophanic Brown Soil, Hewitt 1998; USDA: Dystrochrept, Soil Survey Staff 1998). Soil (0–7.5 cm horizon) was collected in June 2008 from a high country site (43°19′02.88″S; 171°8′31.41″E) on ‘Glenfalloch Station’, a central Canterbury sheep and beef farm in South Island, New Zealand. At an altitude of 740 m a.s.l. and a north-facing aspect, the hill site (25° slope) has had no regular farm management effort towards improving soil fertility, thus minimal fertiliser input since the start of livestock grazing on the property over 100 years ago. At most, a total of 1–2 fertiliser applications of superphosphate have been applied at this site. The soil was prepared by passing through a 4 mm sieve while field moist, removing all plant material, and then mixing thoroughly. Soil analyses were conducted before commencement of the experiment, with results confirming an initial low soil fertility status (Table 5.1).

5.2.2 Treatments

The glasshouse experiment was conducted at the Lincoln University glasshouse facilities, Canterbury, New Zealand. Phosphorus, in the form of Ca(H$_2$PO$_4$)$_4$H$_2$O, was applied at eight different rates; 0, 30, 60, 100, 250, 500, 1000 and 2500 mg P/kg soil. Treatments were replicated four times for each of the six pasture legume species. Basal nutrient addition prior to experiment commencement included sulphur (S) as calcium sulphate (CaSO$_4$) to address the soil plant-available S deficiency, and potassium (K) as potassium chloride (KCl), applied at rates of 500 mg P/kg soil. All pots also received lime at a rate of 4 t/ha equivalent of laboratory grade CaCO$_3$, which corrected soil pH to pH 6.2. The various P treatments and basal nutrients were added to 400 g of the air-dried soil and then mixed thoroughly. The soil was then lightly packed into 0.8 L (10 cm height x 12 cm diameter) pots with saucers, at a bulk density ($\rho_b$) of 0.7 g cm$^{-3}$. De-ionised (DI) water was slowly added to each pot to wet up the soil to a gravimetric water content of 40%. The pots were then moved to the glasshouse and arranged on tables in a randomised block design. The glasshouse was maintained within the range of 10–30 °C, with a mean temperature of 19.9 °C for the duration of the experiment.
Table 5.1 The initial fertility status of an Upland Allophanic Brown soil used for the glasshouse experiment. Topsoil sample (0–75 mm) was collected from a mid-altitude (740 m a.s.l.) central South Island, New Zealand high country site in June 2008.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(^a)</td>
<td>5.2</td>
</tr>
<tr>
<td>Olsen P</td>
<td>11 µg/mL</td>
</tr>
<tr>
<td>Sulphate S(^b)</td>
<td>4 µg/g</td>
</tr>
<tr>
<td>CEC</td>
<td>20 me/100 g</td>
</tr>
<tr>
<td>Base Saturation</td>
<td>39.9%</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>5.7 me/100 g</td>
</tr>
<tr>
<td>Exchangeable Mg</td>
<td>1.59 me/100 g</td>
</tr>
<tr>
<td>Exchangeable K</td>
<td>0.53 me/100 g</td>
</tr>
<tr>
<td>Exchangeable Na</td>
<td>0.11 me/100 g</td>
</tr>
<tr>
<td>Total N</td>
<td>0.63% w/w</td>
</tr>
<tr>
<td>Total C</td>
<td>9.62% w/w</td>
</tr>
<tr>
<td>C/N Ratio</td>
<td>15</td>
</tr>
<tr>
<td>Mineralisable N(^c)</td>
<td>288 kg/ha</td>
</tr>
<tr>
<td>P Retention (ASC)</td>
<td>44%</td>
</tr>
</tbody>
</table>

\(^a\)Measured at 1:2.5 air-dried soil:water ratio  
\(^b\)Late winter soil sample  
\(^c\)Measured by anaerobic incubation (Keeney & Bremner 1966).

5.2.3 Plant establishment and General Methods

‘Natural’ (soil-borne; soil seed bank) seed was allowed to germinate, and these seedlings removed 10–14 days post wetting up of the soil. Seeds of the target pasture legume species were pre-treated by soaking and scarification and then sown directly, using a ‘grid’ sowing pattern using 5 sowing positions and 2 seeds at each position, onto the soil surface in the pots as monocultures, in late September (spring). A small quantity (2mm depth) of soil was sprinkled over the seed and gently packed. Soon after emergence, all legume species were thinned to a final plant density of five plants per pot, equivalent to a field plant density of 450 plants/m\(^2\). The pasture legume species evaluated were: white clover (Trifolium repens, cv. Nomad), subterranean clover (T. subterraneum, cv. Mt Barker), striated clover (T. striatum), suckling clover (T. dubium), cluster clover (T. glomeratum) and, haresfoot trefoil (T. arvense). Seeds of the traditionally sown pasture legume species investigated were sourced commercially. Seed of the naturalised adventive legume species were collected from farmland areas within the South Island. Specifically, T. glomeratum and T. striatum seeds were collected in Canterbury from dryland pasture sites on farmland at Ashley Dene and Aylesbury.
respectively, while *T. arvense* and *T. dubium* were collected from hill country slopes at Mt. Grand Station in Central Otago.

Commercial rhizobia inoculant (Nodulaid, Group B and C, Becker Underwood PTY Ltd.), Group B for all white clover pots and Group C for all annual clover pots, was added 5 weeks after seed germination to insure that an active soil rhizobia population was present. At 5 weeks post germination of seeds, a small quantity of N (30 kg N/ha) nutrient solution, in the form of ammonium nitrate (NH$_4$NO$_3$), was applied to all pots in order to overcome any plant N deficiencies during the seedling establishment phase. Beyond this point, plants were dependant on N sourced from N fixation or soil N for growth. In addition, a nutrient solution (North Carolina State University: Booking 1976; Caradus & Snaydon 1986) containing trace elements was applied on a regular basis (weekly during rapid growth in spring and early summer) to ensure adequate trace element nutrition. Throughout the experiment all pots were watered with DI water daily to maintain gravimetric soil moisture content at 40%, and watered to weight twice weekly.

Herbage harvests of all treatments were conducted in late-November 2008, December 2008, January 2009, February, and March 2009. These harvests represented plant growth at 9, 11, 13, 15, 20 and 27 weeks post germination respectively. The total duration of the experiment was 190 days. Clover plants were harvested by cutting 2 cm from the crown of each plant. Plant material from each pot was then dried at 70°C for 48 hours and weighed for dry matter (DM) yield. Samples were then ground, acid digested (Kjeldahl digest procedure; Blakemore et al. 1987) and analysed for Total P concentration by Molybdenum Blue using a FIA (Flow Injection Analyser; Tecator Inc., Sweden). This information was then combined to give total P uptake for the duration of the experiment. Individual Olsen P tests were run on soil from all pots to determine the level of plant-available P at the conclusion of the experiment (Figure 5.1). Further soil analyses were conducted on a bulked soil sample from all P treatment pots.

### 5.2.4 Statistical analysis

The effects of applied P on plant yield, plant shoot P concentration, plant shoot P uptake and final soil Olsen P was statistically analysed by conducting an analysis of variance (ANOVA) in GenStat 12.2 (Lawes Agricultural Trust, Rothamsted, UK). The model included P treatment rate, pasture legume species and P rate x species interaction as fixed effects. Data were analysed using a randomised complete block design function. Highly significant
interaction effects were observed and therefore regression analysis and curve fitting was undertaken for all species individually in order to better interpret the results.

Figure 5.1 Olsen P values (μg/mL) of a NZ high country soil supplied with increasing levels of P after final legume herbage harvest. Values are means ± SEM (n=24) of Olsen P values from pots across all pasture legume species within each P treatment level (P rate).
5.3 Results

5.3.1 Yield response

Plant growth response to applied soil P differed between pasture legume species, and between P application rates (Figure 5.2, Table 5.2). Mean total accumulated dry matter (TDM) yield of pasture legume species ranged from 0.8–4.4 g DM/pot by the conclusion of the trial (Table 5.2). *Trifolium subterraneum* was the most productive pasture legume species producing 4.4 g DM/pot, followed by *T. arvense, T. repens, T. dubium, T. glomeratum*, and *T. striatum* yielding 3.4, 1.7, 1.4, 1.3 and 0.8 g DM/pot respectively (Table 5.2).

Maximum yields varied between the six species, and the soil phosphorus level at which maximum yields were observed varied also (Figure 5.2 and Table 5.3). Maximum yields ranged from 6.31 g DM/pot for the most productive species *Trifolium subterraneum*, to 1.32 g DM/pot for the least productive species *T. striatum* (Figure 5.2). *Trifolium arvense* was the second most productive legume species yielding 4.3 g DM/pot. *Trifolium repens* produced 2.56 g DM/pot, while *T. glomeratum* and *T. dubium*, produced 1.9 and 1.76 g DM/pot respectively (Figure 5.2).

Three of the legume species (*T. subterraneum, T. repens* and *T. glomeratum*) showed a clear rise to maximum yield, followed by a decline in TDM yield at higher rates of plant-available P in the soil (Figure 5.2). Two legume species (*T. arvense* and *T. dubium*) showed a rapid rise in TDM yields through the lower quartile of soil P levels (0–250 mg P/kg soil), then tapered off to a gentle plateau through to the highest soil P level, with no decline in yield; in the case of *T. dubium*, yield rose again slightly at the highest soil P level (Figure 5.2). *Trifolium striatum* rose quickly to a maximum yield response at 250 mg P/kg soil, then showed a steady declining yield response as soil P level increased to 2500 mg P/kg soil.
Figure 5.2 Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) *Trifolium glomeratum*, (b) *T. arvense*, (c) *T. subterraneum*, (d) *T. dubium*, (e) *T. striatum*, and (f) *T. repens* to increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 2500 mg P/kg soil), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (n=4), with P and $R^2$ values for fitted curve showing data trend.
Table 5.2 Mean values of shoot yield, P concentration and P uptake by six pasture legume species, grown under glasshouse conditions in a NZ high country soil supplied with increasing rates of P (8 levels of P; ranging from 0 to 2500 mg P/kg soil), and mean Olsen P values of soil in which plants were grown.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Shoot Yield (g DM/pot)</th>
<th>Mean Shoot P Concentration (% P)</th>
<th>Mean Shoot P Uptake (mg P/pot)</th>
<th>Olsen P (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. glomeratum</em></td>
<td>1.313</td>
<td>0.405</td>
<td>525.8</td>
<td>81.8</td>
</tr>
<tr>
<td><em>T. arvense</em></td>
<td>3.371</td>
<td>0.399</td>
<td>1412.3</td>
<td>77.3</td>
</tr>
<tr>
<td><em>T. subterraneum</em></td>
<td>4.401</td>
<td>0.588</td>
<td>2405.2</td>
<td>80.5</td>
</tr>
<tr>
<td><em>T. dubium</em></td>
<td>1.440</td>
<td>0.548</td>
<td>808.4</td>
<td>64.3</td>
</tr>
<tr>
<td><em>T. striatum</em></td>
<td>0.810</td>
<td>0.464</td>
<td>378.4</td>
<td>79.2</td>
</tr>
<tr>
<td><em>T. repens</em></td>
<td>1.669</td>
<td>0.394</td>
<td>752.1</td>
<td>77.5</td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td>2.168</td>
<td>0.470</td>
<td>1047.0</td>
<td>76.8</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>0.062</td>
<td>0.021</td>
<td>51.2</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>LSD (5%)</strong></td>
<td>0.173</td>
<td>0.059</td>
<td>143.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P Rate</th>
<th>Total Shoot Yield (g DM/pot)</th>
<th>Mean Shoot P Concentration (% P)</th>
<th>Mean Shoot P Uptake (mg P/pot)</th>
<th>Olsen P (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.264</td>
<td>0.333</td>
<td>426.6</td>
<td>11.2</td>
</tr>
<tr>
<td>30</td>
<td>1.867</td>
<td>0.350</td>
<td>634.6</td>
<td>12.7</td>
</tr>
<tr>
<td>60</td>
<td>1.791</td>
<td>0.360</td>
<td>610.6</td>
<td>16.3</td>
</tr>
<tr>
<td>100</td>
<td>2.123</td>
<td>0.382</td>
<td>812.9</td>
<td>21.1</td>
</tr>
<tr>
<td>250</td>
<td>2.573</td>
<td>0.456</td>
<td>1094.0</td>
<td>46.6</td>
</tr>
<tr>
<td>500</td>
<td>2.815</td>
<td>0.495</td>
<td>1406.2</td>
<td>84.3</td>
</tr>
<tr>
<td>1000</td>
<td>2.524</td>
<td>0.612</td>
<td>1601.0</td>
<td>150.6</td>
</tr>
<tr>
<td>2500</td>
<td>2.385</td>
<td>0.743</td>
<td>1790.5</td>
<td>271.3</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>0.071</td>
<td>0.024</td>
<td>59.1</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>LSD (5%)</strong></td>
<td>0.199</td>
<td>0.068</td>
<td>165.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><em>P</em> (significance)</th>
<th>Species</th>
<th>P Rate</th>
<th>Sp*P Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

1Olsen P values at the completion of the experiment.
*** Significant @ < 0.1 % level

The soil P level at which maximum yield was observed also varied between the six pasture legume species (Table 5.3). *Trifolium glomeratum, T. subterraneum* and *T. repens* achieved maximum yield response at the low–intermediate level of 500 mg P/kg soil, while *T. arvense* and *T. striatum* showed maximum yield response at the lower soil P level of 250 mg P/kg soil. *Trifolium dubium* produced maximum yield at 2500 mg P/kg soil; the highest soil P level, in contrary to all the other species in this trial (Figure 5.2).
Table 5.3  Rate of P application and shoot P concentration at which maximum yield was observed for each pasture legume species.

<table>
<thead>
<tr>
<th>Species</th>
<th>P Rate (mg P/kg soil)</th>
<th>Maximum yield (g DM/pot)</th>
<th>Shoot P concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. glomeratum</td>
<td>500</td>
<td>1.9</td>
<td>0.44</td>
</tr>
<tr>
<td>T. arvense</td>
<td>250</td>
<td>4.3</td>
<td>0.38</td>
</tr>
<tr>
<td>T. subterraneum</td>
<td>500</td>
<td>6.3</td>
<td>0.61</td>
</tr>
<tr>
<td>T. dubium</td>
<td>2500</td>
<td>1.7</td>
<td>0.96</td>
</tr>
<tr>
<td>T. striatum</td>
<td>250</td>
<td>1.3</td>
<td>0.46</td>
</tr>
<tr>
<td>T. repens</td>
<td>500</td>
<td>2.6</td>
<td>0.47</td>
</tr>
</tbody>
</table>

5.3.2  Shoot P concentration

Pasture legume species showed the general pattern of increasing P concentration in shoots as P availability increased in the soil (Figure 5.3); the one exception to this trend was *T. striatum*. Shoot P concentration steadily increased at a similar rate from 0–2500 mg P/kg soil for *T. arvense, T. subterraneum, T. dubium* and *T. repens*, while P concentration of *T. glomeratum* shoots rose more rapidly between 0–250 mg P/kg soil before flattening off and steadily increasing in a similar pattern to the other species. *Trifolium striatum* shoot P concentration was relatively high at the natural soil P level (no added P) but showed a general decline and then minimal change as soil P levels increased; this trend was weakest out of all the other species ($R^2=0.64$), (Figure 5.3). Mean shoot P concentration was highest for *T. subterraneum* and *T. dubium*, intermediate for *T. striatum* and lowest for *T. glomeratum, T. arvense* and *T. repens* (Table 5.2).

However, the relationship between shoot P concentration and TDM yield varied between the pasture legume species. The % P concentration in shoots at which maximum yield response was achieved for *T. glomeratum, T. arvense, T. subterraneum, T. dubium, T. striatum* and *T. repens* was 0.46, 0.62, 0.64, 0.96, 0.35 and 0.5 respectively (Figure 5.4). *Trifolium subterraneum* showed a clear rise to maximum yield followed by a steady yield decline as shoot P increased above 0.64%. 

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Figure 5.3 Comparison of shoot P concentration of pasture legume species (a) T. glomeratum, (b) T. arvense, (c) T. subterraneum, (d) T. dubium, (e) T. striatum, and (f) T. repens grown in 4 mm-sieved NZ high country soil supplied with increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 2500 mg P/kg soil). Data are mean values ± SEM (n=4), with P and $R^2$ values for fitted curve showing data trend.
Figure 5.4 Comparison of Total DM yield in relation to shoot P concentration (%) of pasture legume species (a) *T. glomeratum*, (b) *T. arvense*, (c) *T. subterraneum*, (d) *T. dubium*, (e) *T. striatum*, and (f) *T. repens* grown in 4 mm-sieved NZ high country soil supplied with increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 2500 mg P/kg soil). Data are mean values ± SEM (*n*=4), with *P* and $R^2$ values for fitted curve showing data trend.
5.3.3 P uptake

The nature of P uptake varied between the six pasture legume species. The mean level of P uptake ranged from 378.4 mg P/pot in *T. striatum* plants up to 2405.2 mg P/pot in *T. subterraneum* plants (Table 5.2). *Trifolium glomeratum*, *T. subterraneum*, and *T. repens* all showed a rise to a maximum P uptake level, while the level of P uptake continued to rise for *T. arvense* and *T. dubium*. In contrast, P uptake by *T. striatum* generally declined with increasing levels of soil P (Figure 5.5). Maximum P uptake was observed at 1000 mg P/kg soil in *Trifolium glomeratum*, *T. subterraneum*, and *T. repens* plants. The pattern of P uptake for *T. arvense* and *T. dubium* plants showed to rapidly increase from 0 up to 100 mg P/kg soil, then changed to a gentle increase from 100 mg P/kg soil up to the highest P rate of 2500 mg P/kg soil.

5.3.4 P-response efficiency

The P rate at which highest P-response efficiency was observed also varied among species (P rate x Sp interaction, *P*<0.001) (Table 5.2). *Trifolium glomeratum*, *T. arvense*, *T. subterraneum*, and *T. repens* all showed greatest P-response efficiency at the lowest soil P level above 0 mg P/kg soil (control rate), namely 30 mg P/kg soil (Figure 5.6). In contrast, highest P-response efficiency for *T. dubium* and *T. striatum* occurred at the higher soil P level of 100 mg P/kg soil. In general, the order of greatest P-response efficiency by species was as follows: *T. subterraneum* > *T. arvense* > *T. repens* > *T. glomeratum* > *T. dubium* > *T. striatum* (Figure 5.6).
Figure 5.5 Comparison of total P uptake by pasture legume species (a) *T. glomeratum*, (b) *T. arvense*, (c) *T. subterraneum*, (d) *T. dubium*, (e) *T. striatum*, and (f) *T. repens* grown in 4 mm-sieved NZ high country soil supplied with increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 2500 mg P/kg soil). Data are mean values ± SEM (n=4), with p and $R^2$ values for fitted curve showing data trend.
Figure 5.6 Comparison of P-response efficiency of pasture legume species (a) *T. glomeratum*, (b) *T. arvense*, (c) *T. subterraneum*, (d) *T. dubium*, (e) *T. striatum*, and (f) *T. repens* grown in 4 mm-sieved NZ high country soil supplied with increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 2500 mg P/kg soil). Data are mean values ± SEM (n=4), with P and $R^2$ values for fitted curve showing data trend.
5.4 Discussion

The response to P application by the six pasture legume species (five annual and one perennial species) showed significant differences in terms of total DM yield, P uptake, P-response efficiency and shoot P concentration between species. The key results, and implications for field conditions, are discussed below.

5.4.1 Yield performance of pasture legume species to increasing soil P

Total DM yields differed between species, with \textit{T. subterraneum} being the highest producing legume, followed by \textit{T. arvense}, \textit{T. repens}, \textit{T. dubium} and \textit{T. glomeratum}, with \textit{T. striatum} being the least productive legume (Figure 5.2 and Table 5.2). The naturalised, adventive annual \textit{Trifolium arvense} out yielded the perennial \textit{T. repens}, cv. Nomad at every soil P level (Figure 5.2). This suggests that \textit{Trifolium arvense} may have a stronger agronomic potential than \textit{T. repens}, cv. Nomad, a cultivar selected for increased persistence in dry environments prone to low summer moisture levels (www.agricom.co.nz). \textit{Trifolium glomeratum} and \textit{T. dubium} yields did not differ significantly from each other, though both out yielded \textit{T. striatum} as soil P level increased above 100 mg P/kg soil.

The superior yield performance of \textit{T. subterraneum}, cv. Mt Barker can be explained by its large seed size, with greater endosperm reserves relative to all the other species. This enabled rapid early seedling development, and subsequent exhibition of its good agronomic potential, under the optimum soil and climatic glasshouse conditions, subject to soil P level treatments. Caradus (1980) reported on the responsiveness of \textit{T. repens}, cv. Huia, \textit{T. subterraneum}, cv. Woodenellup, \textit{T. striatum}, \textit{T. arvense}, and \textit{T. dubium} to added P (phosphoric acid), in terms of shoot dry weight and total shoot P content, grown at comparable P rates to this study, of 300 and 2000 mg P/kg soil in pots of naturally P-deficient soil (Stratford coarse sandy loam) over 24 weeks. \textit{Trifolium repens}, cv. Huia yielded 1.7 and 5.3 g dry weight at 300 and 2000 mg P/kg soil respectively, while \textit{T. subterraneum}, cv. Woodenellup yielded 3.5 and 6.8 g, \textit{T. striatum} 1.1 and 1.7 g, \textit{T. arvense} 1.0 and 2.3 g, and \textit{T. dubium} 2.1 and 3.7 g at 300 and 2000 mg P/kg soil respectively. Similarly to this study, \textit{T. striatum} was the least P responsive annual legume species. The yield results for \textit{T. arvense} and \textit{T. dubium} however contrast our results, as \textit{T. arvense} yielded less and \textit{T. dubium} yielded more. This was possibly due to differences in germplasm used for these species. \textit{Trifolium subterraneum} was the most productive species across all P rates. Differences in cultivar of \textit{T. subterraneum} and \textit{T. repens} grown by Caradus (1980) to those in our study may explain the contrasting trends in yield.
responses between the two comparable P rates; both species yielded greater at 2000 mg P/kg soil than at 300 mg P/kg soil which contrasts strongly to yield response trends for these species in our study. Additionally, the volcanic soil examined by Caradus (1980) contrasts with the sedimentary high country Allophanic Brown soil of this study, in terms of soil parent material (high allophane content / very high P retention capacity), and environmental soil-forming conditions.

Blair & Cordero (1978) also found that *T. subterraneum* out-yielded *T. glomeratum* over the entire range of P applied, equivalent to 0–80 mg P/kg soil, in a 10 week pot trial. Caradus et al. (1995) found the average shoot dry weight of 119 *T. repens* cultivars increased, and were significantly different, with increasing level of P supply. Rates of P ranged from 0–500 mg P/kg soil, with the increase in shoot dry weight from 400 to 500 mg P/kg soil being not significant. This is comparable to the present study, where the maximum yield response of *T. repens*, cv. Nomad was achieved at 500 mg P/kg soil, with the yield response decreasing as the P rate increased beyond this point.

Hart & Jessop (1984) examined the growth responses to P of *T. repens*, cv. Huia and *T. dubium* Sibth growing in soil from the B Horizon of Egmont sandy loam; a very N deficient soil of high phosphate fixing capacity. Phosphorus was added to the soil in the form of H₃PO₄ at levels equivalent to 50, 100, 250, 500, 1000 and 2000 mg P/kg soil. In accord with the results of this glasshouse study, they observed *T. repens* responding more strongly to added P than *T. dubium*, with the latter having lower shoot dry weights. Steepest increases in shoot dry weight of both species occurred at low P rates (0–500 mg P/kg soil). They concluded *T. dubium* to be a species that has a relatively small response to improvements in P availability, which is in agreement with results of this experiment.

Dodd & Orr (1995) investigated the phosphorus response of 18 herbaceous annual legume species in an acidic (pH 5.4), low P fertility (Olsen P range 6–10 µg/mL), high P retention soil (90–95%) soil, subjected to two P rates (0 and 0.42 g P/soil core); the latter rate being equivalent to an Olsen P of 24 µg/mL. The species used in common to this study were *T. subterraneum*, cv. Mt Barker and *T. arvense*, with both species showing a positive dry matter yield response to added P. They stated that *T. arvense* was more responsive to added P than *T. subterraneum*, contrary both to what this study, and Caradus (1980) reported. Again, the soil parent material (high allophane content / very high P retention capacity) may explain the difference in results.
In this glasshouse study, the soil P level at which maximum yield was observed varied between species (Figure 5.2). This result suggests that the optimum P requirement of these species is different. Maximum/peak yield response for *T. subterraneum*, *T. glomeratum* and *T. repens* occurred at an intermediate soil P level of 500 mg P/kg soil (Olsen P of 84 µg/mL) (Table 5.2) equivalent to 221 kg P/ha. Beyond this point however, these three species were unable to utilize further increases in available P for shoot growth. This result suggests that factors other than plant-available soil P were limiting yield beyond P application rates of 500 mg P/kg soil. *Trifolium dubium* maximum yield was observed at the highest soil P level treatment (2500 mg P/kg soil, Olsen P of 271 µg/mL; Table 5.2) suggesting this naturalised annual legume species has the ability to take up P available in the soil at very high levels. However, the difference between mean DM yield produced at 0 mg P added/kg soil (Olsen P 11 µg/mL) and that produced at 2500 mg P added/kg soil (Olsen P 271 µg/mL) was only 0.63 g. This species appears to be able to continue to take up P over a wide range of availability, though remaining very unresponsive to P in terms of DM yield, suggesting perhaps a very low P requirement before being able to manifest its growth potential. *Trifolium dubium* is a prolific seed producer and may translocate stored P in its shoots into reproductive plant parts for seed production.

These results suggest that critical P requirement of *T. subterraneum*, *T. glomeratum* and *T. repens* is 500 mg P/kg soil. This would be equivalent to a phosphate fertiliser rate of 221 kg P/ha. *Trifolium striatum* was the least productive pasture legume species with highest total yield being obtained at 250 mg P/kg soil (Figure 5.2) suggesting a lower critical P requirement. *Trifolium striatum* presence and abundance has been associated with low soil P levels (Beale et al. 1993; Maxwell et al. 2010). In contrast, the maximum/peak yield for *T. arvense*, which was the second most productive species, also occurred at 250 mg P/kg soil (Olsen P of 47 µg/mL), reflecting a more efficient P uptake and perhaps utilisation ability at lower available soil P levels compared with that of *T. repens*, *T. glomeratum*, and *T. dubium* (Figure 5.5 and 5.6). Critical P requirement for *T. arvense* in the field, based on peak yield response in this glasshouse experiment occurring at 250 mg P/kg soil, would be an Olsen P of 47 µg/mL; or 111 kg P/ha equivalent.
5.4.2 Change in shoot P concentration with increasing P rate

In contrast to all other species, the shoot P concentration of *Trifolium dubium* was greatest (0.96% P) within herbage grown at the highest soil P level (Figure 5.3); the point at which maximum yield response occurred (Figure 5.2). However, it was relatively unresponsive to P with only small yield increases with increasing levels of soil P; a yield range of 0.63 g DM/pot. Hart & Jessop (1984) found *T. dubium* Sibth produced lower shoot dry weights than both *Lotus pedunculatus* Cav., cv. Maku and *T. repens*, cv. Huia at all P treatment levels, and stated it has a relatively small response to increasing P availability. Very minimal to no correlation between total accumulated DM yield and shoot P concentration was observed for *T. dubium* and *T. striatum*, suggesting these two legume species produce similar yields across all levels of shoot P (Figure 5.4).

In general all pasture legume species, with the exception of *T. striatum*, showed steadily rising shoot P concentrations with increased availability of soil P (Figure 5.3). Hart & Jessop (1984) reported leaf P concentration of *T. dubium* Sibth and *T. repens*, cv. Huia rose with P supply. Similarly, Blair & Cordero (1978) found that *T. subterraneum* and *T. glomeratum* showed increases in shoot P concentration with increasing rates of applied P. This is in accord with our observations. However, the observed increase in shoot P concentration continued beyond the point of maximum yield response for *T. glomeratum*, *T. arvense*, *T. subterraneum* and *T. repens* (Figure 5.2), indicating these species continued to take up P from the soil without showing a corresponding yield increase, suggesting ‘luxury’ P uptake by these species.

The shoot P concentration at which maximum yield was observed varied between the pasture legume species. There was a steady linear increase in the relationship between total DM yield and shoot P concentration for *T. repens* and *T. subterraneum* to the point of maximum yields at 0.47 and 0.64 % P respectively, with yields decreasing at higher shoot P concentrations (Figure 5.4). In comparison, the nature of total DM yield increase in relation to shoot P concentration for *T. glomeratum* and *T. arvense* was curvilinear, with maximum yields occurring at 0.45 and 0.38 % P respectively.
5.4.3 P uptake pattern within species

The P uptake varied between the species, with three species (*T. glomeratum*, *T. subterraneum*, and *T. repens*), showing a similar profile of P uptake rising to a maximum at soil P level of 1000 mg P/kg soil. In contrast, the P uptake profiles of *T. arvense* and *T. dubium* continued to increase, though changing between 100–250 mg P/kg soil, with the rate of P uptake slowing down but continuing to steadily increase up to 2500 mg P/kg soil (Figure 5.5). This result suggests that these two species had luxury P uptake, while *T. glomeratum*, *T. subterraneum*, and *T. repens* have a limitation in their ability to utilise high rates of available P for producing shoot biomass. There are no reports suggesting P toxicity causes a reduction in DM yields of pasture legumes grown under glasshouse conditions however. The magnitude of P uptake varied between the clover species, with *T. subterraneum* showing the greatest mean level of P uptake from the soil across all P rates, at 2405.2 mg P/pot (Table 5.2) and maximum P uptake of 4114 mg P/pot at 2500 mg P/kg soil (Figure 5.5). This is reflected in the TDM yield response of *T. subterraneum* being 130% greater than *T. arvense*, which had the second highest P uptake and yield response (Table 5.2). *Trifolium striatum* was again the species that showed to be most conservative and poorly responsive to high levels of P in the soil.

5.4.4 P-response efficiency; determining the ‘critical’ P use of species

The ability to acquire P from the soil and use it efficiently for biomass production is an important characteristic for adaptation to soils low in available P (Pang et al. 2010). We calculated P-response efficiency for each species using the yield response results obtained in this experiment to determine the ability of each species to produce biomass at a given level of available P in the soil; essentially, determining how much P was required for each gram of DM produced. In general *Trifolium subterraneum* was the most efficient species in utilising applied P for biomass production, followed by *T. arvense* (Figure 5.6). For P supply between 0 and 30 mg P/kg soil, *T. repens* was third best species at using applied P. Beyond the level of 30 mg P/kg soil however, *T. repens* became the least efficient out of the top three higher-yielding clover species, including *T. subterraneum* and *T. arvense*, and was less efficient than *T. glomeratum* at P rates above 30 mg P/kg soil, indicating *T. glomeratum* was better at using lower soil P levels and has a lower P requirement to produce DM.
5.4.5 Implications for low input extensive grazing systems

Although *Trifolium repens* has traditionally been, and still is, the most widely sown legume species for pasture improvement in NZ hill and high country areas, its primary limitation of poor persistence in summer dry environments (400–700 mm rainfall/annum) make it increasingly less desirable as a management option for the progressive hill and high country pastoral farmer. Such farmers aim to increase legume abundance in extensive hill country through broadcast over-sowing onto low fertility middle and lower altitude slopes. This glasshouse study has revealed the yield responses of four naturalised annual legume species to increasing levels of added P in a high country soil in comparison to the yield responses of two traditional legume species used in pasture improvement in hill and high country summer dry areas.

Gaining understanding of P requirements of plant species is important for the purposes of introduction, selection and breeding (Pang et al. 2010). *Trifolium arvense* shows the most promise as a species to be utilised, and its spread and abundance increased/encouraged in extensive hill and high country ecosystems, with a critical P requirement for maximum yield lower than *T. repens*, cv. Nomad and *T. subterraneum*, cv. Mt Barker at 250 mg P/kg soil; equivalent to a field application of 111 kg P/ha. *Trifolium arvense* was almost twice as productive as *T. repens* under glasshouse conditions on this soil, and had greater P response efficiency at lower soil P levels; at the same shoot P concentration of 0.4%, *T. arvense* produced nearly twice the DM as *T. repens*. Such an agronomic difference is potentially beneficial in the field as being an annual pasture species it has the ability to complete its life cycle before soil moisture deficits occur in late spring-early summer in summer-dry hill country areas. Although being less productive than *T. subterraneum* in this study, the herbage DM production of *T. arvense* could be improved by breeding and selection of better performing cultivars, and searching to find ecotypic variation (Pang et al. 2010) within the large areas of NZ hill and high country that *T. arvense* is found to survive and exist. *Trifolium glomeratum* also shows promise with a comparable yield P response trend and greater P response efficiency than *T. repens* as soil P rates increase in the lower quartile of the P rate treatments used in this trial.

The results of this 27-week glasshouse study need to be examined further in long-term field studies. Such studies, conducted under field climatic and environmental conditions, should identify how the more P responsive naturalised species such as *T. arvense* and *T. glomeratum*, when grown as monocultures or mixtures, perform when subjected to realistic P fertiliser rates.
in the field. As future long-term sustainability of hill and high country pasture areas may depend on species with greater P use efficiency and production sustained with lower inputs of fertiliser P, it will be to the benefit of hill and high country pastoral farmers if these naturalised species are identified as being able to use P already present in soil more efficiently for herbage production, thus reducing the requirement for higher fertility and hence fertiliser P inputs to the system. Furthermore, determining the extent to which optimising soil fertility for legume growth in hill country grassland can elevate N inputs towards allowing near-maximum production of pasture requires more research to quantify N inputs derived from pasture legume N fixation (Bowatte et al. 2006).

5.5 Conclusions

*Trifolium subterraneum*, cv. Mt Barker was the most P responsive legume species with the largest magnitude difference in yield DM response, shoot P concentration, P uptake, and P response efficiency compared to all the other species. *Trifolium arvense* was the most P responsive naturalised annual species, and showed greater yield shoot DM response and P response efficiency than the perennial *T. repens*, cv. Nomad. *Trifolium glomeratum* was intermediate in yield response though more efficient at utilising P at low levels for DM production than *T. repens*, cv. Nomad. *Trifolium dubium* and *T. striatum* were the least P responsive legume species. The results suggest that naturalised annual pasture legume species (*T. arvense* and *T. striatum*) have lower P requirements for maximum yield response than *T. repens* and *T. subterraneum*, and that *T. arvense* shows potential for further P response investigation under field conditions. Germplasm improvement and subsequent introduction into low fertility, summer dry hill and high country grassland is required to promote the spread of a N-fixing species already existing in such agroecosystems.
Chapter 6
Sulphur and lime response of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions


6.1 Abstract
Adventive annual clovers play a critical role in nitrogen (N) cycling and feed quality in extensive summer-dry hill country, where traditionally sown white and subterranean clovers often fail to persist. However, very little is known about the edaphic (e.g. soil fertility) requirements of these species. The growth response and nutrient uptake of four adventive annual clovers to applied sulphur (S) or lime, grown in a typical low fertility South Island (S.I.) high country soil, were investigated under glasshouse conditions and compared to white and subterranean clovers as reference species. The annual species had yield responses of 12–17%, or were unresponsive, to S applications. Trifolium repens and T. striatum responded to liming at low lime rates, while all other species had negative yield responses to liming. Maximum yields were generally in the order of T. subterraneum ≥ T. arvense > T. striatum ≥ T. dubium > T. glomeratum > T. repens. For lime treatments, yields were strongly driven by phosphorus (P) availability, linked to soil pH. The data indicate that the adventive annual clovers are better adapted to low soil fertility (low pH and S) conditions, which in turn may be an important factor contributing to their success under S.I. high country field conditions.

Keywords: annual clovers; sulphur; lime; soil pH; yield response; Al toxicity; high country.

6.2 Introduction
The productivity of New Zealand high and hill country is strongly driven by nitrogen (N) inputs from sward legumes (Haynes & Williams 1993). An important component of pastoral intensification is to increase legume abundance, so as to provide increased N inputs and feed to N deficient, high country grassland (Boswell et al. 2007). However, the establishment and
persistence of sown clover species such as white clover (*Trifolium repens*) and subterranean clover (*T. subterraneum*) is often limited in summer dry, low soil fertility hill country areas (Knowles et al. 2003; Power et al. 2006). This is in contrast to the common presence of other naturalised, adventive and unsown clovers, such as cluster clover (*T. glomeratum*), haresfoot trefoil (*T. arvense*), striated clover (*T. striatum*) and suckling clover (*T. dubium*) (Power et al. 2006) that may be more suited to the low soil fertility and microclimates that exist on these hill slopes (Boswell et al. 2003; Maxwell et al. 2010). In addition, the sustainability of hill and high country pastoralism in New Zealand is currently under considerable pressure, resulting from central government policy to retire significant areas of high altitude land (>1000 m a.s.l.) to improve indigenous grassland conservation and recreation resources (Barratt et al. 2006; Brower 2008). This has increased the need to improve pastoral primary productivity of the remaining middle to lower altitude land (900–300 m a.s.l.) used for fine wool and meat (lamb and beef) production from extensive livestock grazing agroecosystems (O’Connor 2003; Barratt et al. 2006).

Much research has been conducted investigating white clover (*T. repens* L.) dry matter (DM) and growth responses to added P in New Zealand (Caradus & Snaydon 1986; Caradus et al. 1995). As such, highly productive clover species and pastures are generally adapted to high fertility soil conditions and do not perform well in infertile and/or acid soil conditions (Haynes & Williams 1993; Moir et al. 2000). ‘Improved’ pastures therefore require annual maintenance fertiliser inputs, such as single superphosphate (SSP) to elevate soil P and S fertility and sustain high production (Moir et al. 1997). In contrast, annual fertiliser inputs to these farming systems are, in reality, low, driven by the economics of fertiliser inputs. As a result, many high and hill country soils in New Zealand have soil fertility levels (soil pH, plant-available P and S) far below optimum for many common pasture legume species, such as white clover (*T. repens*). Moreover, little research has been conducted looking at the growth, dry matter production and nutrient uptake of naturalised adventive annual pasture legume species (Boswell et al. 2003).

Research examining the effects of soil S fertility and liming on pasture legumes in New Zealand is limited. In terms of S, most researchers have conducted field trials focusing on relationships between total sward growth and S fertility (Wheeler & Thorrold 1997; Morton et al. 1999a; Smith et al. 2004; Craighead & Metherell 2006). Other field studies (Mackay et al. 1988; Sinclair et al. 1996; Morton et al. 1998; Jarvis et al. 1998) have considered the S/legume relationship, but have often involved only one clover species (*T. repens* L. or *T.*
ambiguum Bieb.). Boswell et al. (2007) reported large S responses by *T. arvense* and, in particular, large decreases in N fixation, where S fertiliser was not applied. Detailed climate controlled experiments examining single clover species growth and nutrient uptake response to S fertility status are scarce. Some limited data is available on the relationships between S and *T. subterraneum* (Gilbert & Robson 1984) and *T. repens* (Anderson et al. 1998). Considering that S deficiencies are widespread on South Island soils (Edmeades et al. 2005), experiments providing detailed species-specific pasture legume S response data are required.

Studies examining soil pH changes due to lime addition and associated changes in soil exchangeable aluminium (Al) in South Island high country soils are scarce (Moir & Moot 2010). Aluminium at toxic levels interferes with the uptake, transport and utilisation of essential elements for plants, adversely affects photosynthesis and yield (Roy et al. 1988), and impairs root elongation and development (Barcelo & Poschenrieder 2002), resulting in impaired nutrient and water uptake and thus, drought stress susceptibility (Samac & Tesfaye 2003). Several field trial studies have provided information on total DM response (Edmeades et al. 1984b; Shannon et al. 1984) or legume DM response (Lambert & Grant 1980; Edmeades et al. 1983, 1990; Wheeler et al. 1997) to liming. Annual pasture legume response to liming has been investigated with limited scope, and under field conditions only (Edmeades et al. 1990; Scott & Cullis 1992; Hayes et al. 2008). Glasshouse studies investigating relationships between liming and annual pasture legumes have not been conducted, and only partial information is available for a narrow range of pasture legumes under glasshouse conditions (Wood et al. 1984; Wood & Cooper 1985; Edmeades et al. 1991). Identifying the limiting factors (Al toxicity and/or macro and micronutrient deficiency) that affect clover growth on a given soil, either singly or collectively, can have a large impact on determining the lime requirement for remedial action (Edmeades & Ridley 2003). With the suite of naturalised adventive annual clovers that are adapted to the dry and semi-arid grasslands in New Zealand (Boswell et al. 2003), more knowledge, both ecological and agronomic, regarding such clover species is therefore required.

This experiment examined the vegetative dry matter response of four (non-commercial) naturalised, adventive species (cluster, haresfoot, suckling and striated), one commercial annual species (subterranean clover) and one commercial perennial species (white clover) to added S or lime in a South Island high country soil, grown under glasshouse conditions. This study is part of a larger suite of field and climate-controlled experiments examining the role of adventive annual clovers in extensively grazed high and hill country environments.
6.3 Materials and methods

6.3.1 Soil sampling and seed

The soil used was a ‘Kaikoura Series’ High Country Southern Brown Soil (NZ classification: Upland Allophanic Brown Soil (Hewitt 1998); United States Department of Agriculture classification: Dystrochrept (USDA 1998). Soil (0–7.5 cm horizon) was collected within an altitude range of 650–800 m a.s.l. from a moderately steep (30°) north-facing hill slope on ‘Glenfalloch Station’ (43°18'54.56"S; 171°11' 45.91" E) in February 2009. Historical fertiliser inputs at this hill site are low, at 4 kg of P and 5 kg S/ha/year for the last 100 years. Collected soil (field moist) was bulked, passed through a 4 mm sieve; soil analyses were conducted (Blakemore et al. 1987) and the results indicated an initial low soil fertility status (Table 6.1).

The clover species evaluated were white clover (Trifolium repens L. cv. Nomad), subterranean clover (T. subterraneum L. cv. Mt Barker), striated clover (T. striatum L.), suckling clover (T. dubium Sibth), cluster clover (T. glomeratum L.) and haresfoot trefoil (T. arvense L.). Seeds of the traditionally sown clover species investigated were sourced commercially. Seed of the naturalised, adventive clover species were collected from farmland areas within the South Island. Specifically, T. glomeratum and T. striatum seeds were collected in Canterbury from dryland pasture sites on farmland at Burnham and Aylesbury respectively, while T. arvense and T. dubium were collected from hill country slopes at Mt. Grand Station in Central Otago.
Table 6.1 Initial fertility status of an Acidic Allophanic Brown soil used in the glasshouse experiment. Topsoil sample (0–75 mm) was collected from a mid-altitude (650–800 m) central South Island, New Zealand high country site in February 2009.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(^a)</td>
<td>5.2</td>
</tr>
<tr>
<td>Olsen P</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Sulphate S(^b)</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>Organic S</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>CEC</td>
<td>20 cmol/kg</td>
</tr>
<tr>
<td>Base Saturation</td>
<td>41.2%</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>6.1 (20 cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Mg</td>
<td>1.39 (20 cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable K</td>
<td>0.6 (20 cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Na</td>
<td>0.06 (20 cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Al</td>
<td>3.7 mg/kg</td>
</tr>
<tr>
<td>Total N</td>
<td>0.55% w/w</td>
</tr>
<tr>
<td>Total C</td>
<td>7.46% w/w</td>
</tr>
<tr>
<td>C/N Ratio</td>
<td>13.6</td>
</tr>
<tr>
<td>Mineralisable N(^c)</td>
<td>256 kg/ha</td>
</tr>
<tr>
<td>P Retention (ASC)</td>
<td>45%</td>
</tr>
</tbody>
</table>

\(^a\) Measured at 1:2.5 air-dried soil:water ratio  
\(^b\) Late summer soil sample  
\(^c\) Measured by anaerobic incubation (Keeney and Bremner 1966)

6.3.2 Treatments

Soils received either S or lime treatments. Sulphur, as laboratory grade CaSO\(_4\) (gypsum) was applied at seven different treatment rates; 0, 10, 20, 30, 60, 120 and 240 mg S/kg soil (equivalent to 0, 5, 11, 17, 33, 66, 132 kg S/ha). Treatments were replicated four times for each of the six clover species. Basal lime (laboratory grade CaCO\(_3\), at a rate of 4 t/ha equivalent) was also incorporated into the soil. Mean soil pH level at the conclusion of the experiment was 6.1. The S treatments and basal lime were added to 400 g of the air-dried soil, mixed thoroughly and packed into 0.8 L (10 cm height x 12 cm diameter) pots at a \(\rho_b\) (bulk density) of 800 kg/m\(^3\). Upon transfer of pots to the glasshouse, 300 mL of de-ionised water was slowly added to each pot to wet up the soil to an initial gravimetric water content of 40%. Pots were then arranged on tables in a randomised block design. During the experiment, pots were regularly watered to weight, maintaining gravimetric water content at 40%. The glasshouse was maintained within the temperature range of 8–33 ºC with a mean temperature of 18.2 ºC for the duration of the experiment. Basal macro and micro nutrients were supplied
to all pots for the duration of the experiment. A nil S (and N), ‘semi-complete’ modified nutrient solution (North Carolina State University: Booking 1976; Caradus & Snaydon 1986) was added to all pots at a rate of 90 mL nutrient solution per pot per week. The weekly supply of macro and micro nutrients from the nutrient solution applied the equivalent of 250 kg P/ha, 650 kg K/ha and 40 kg Mg/ha during the experiment, with adequate quantities of trace elements.

Before the commencement of the experiment, the soil was analysed in the laboratory to establish the pH buffering capacity (pH buffering curve) for this soil type. Appropriate treatment liming rates were then selected based on the buffering curve, and the associated projected soil pH changes with lime additions. For lime treatments, laboratory grade CaCO$_3$ was added to soil at rates of 0, 1, 2, 4 and 8 t lime/ha equivalent, and set up in the same manner as the S treatment pots. The weekly supply of macro and micro nutrients from the nutrient solution was identical to the S treatment pots, but in addition, contained S (112 kg S/ha equivalent during the experiment).

6.3.3 Plant Establishment and General Methods

The pot trial was established in the first week of May 2009. ‘Natural’ (soil-borne; soil seed bank) seed was allowed to germinate, and these seedlings removed 10–14 days post wetting up of the soil. Seeds of the target clover species were pre-treated by soaking and scarification and then broadcast sown (10 seeds/pot) onto the soil surface in the pots as monocultures. A small quantity (2–3 mm depth) of soil was sprinkled over the seeds and gently packed. Soon after germination, all clover species were thinned to a final plant density of 5 plants/pot; equivalent to a field plant density of 450 plants/m$^2$.

A commercial rhizobia inoculant (Nodulaid, Group B and C, Becker Underwood PTY Ltd.) Group C was added to all annual clover pots, and Group B to all white clover pots, two days after seed sowing to ensure that an active soil rhizobia population was present. At 3 weeks post germination of seeds, a small quantity of N (25 kg N/ha) nutrient solution, in the form of ammonium nitrate (NH$_4$NO$_3$) was applied to all pots in order to overcome any plant N deficiencies during the seedling establishment phase. Beyond this point, plants were dependant on N sourced from N fixation or soil N for growth.
Herbage harvests were conducted in August, September, November and December 2009. These harvests represented plant growth at 10, 13, 17, 23 and 29 weeks post germination respectively. The total duration of the experiment was 232 days. Clover plants were harvested by cutting 2 cm from the crown of each plant. Plant material from each pot was then dried at 70 °C for 48 hours and weighed for dry matter (DM) yield. Herbage samples were then finely ground. A 0.1000 g proportion of each ground sample was digested in 2 mL of nitric acid (HNO$_3$) on a heating block at 110°C for 2 hours and then topped up to 5 mL with deionised water in preparation for analysis. Digest samples were then analysed for a complete range of elements (excluding N) using ICP-OES analysis (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). This information was then combined to give total S uptake by plants from S treatment pots, for the duration of the experiment. At the conclusion of the experiment, individual sulphate (SO$_4^{2-}$)-S tests were conducted on soil from all S treatment pots, and individual soil pH readings were conducted on all lime treatment pots, to determine the levels of plant-available S (sulphate-S), and the pH levels of soil.

### 6.3.4 Statistical analysis

The effects of applied S on clover yield, shoot S concentration, S uptake, and final soil SO$_4^{2-}$-S, and the effects of applied lime on clover yield and clover shoot macro and micro nutrient concentrations, was statistically analysed by conducting analysis of variance (ANOVA) in GenStat 13 (Lawes Agricultural Trust, Rothamsted, UK). The model for analysing S treatments included S treatment rate, clover species, and S rate x species interaction as fixed effects, while the model for analysing lime treatments included lime treatment, clover species, and lime rate x species interaction as fixed effects. Data were analysed using a randomised complete block design function. Highly significant ($P<0.001$) interaction effects were observed and therefore regression analysis and curve fitting was undertaken for all species individually in order to better interpret the results.
6.4 Results

6.4.1 S Yield response

The individual clover species had low response or were unresponsive to added S (Figure 6.1), though S addition did have a significant overall influence on yield ($P<0.05$, Table 6.2). For species that were S responsive, the yield response occurred at low S application rates. A 13–16.5% increase in total accumulated dry matter (TDM) yield was observed for *T. dubium*, *T. arvense* and *T. striatum*. Yield increases of 12.9% for *T. dubium* and 14.8% for *T. arvense* were observed between 0 and 30 mg S/kg soil, while a yield increase of 16.5% was observed for *T. striatum* between 0 and 60 mg S/kg soil. Surprisingly, *T. repens* cv. Nomad was unresponsive, showing a small decline in TDM yield with increasing S rate (Figure 6.1). The soil S levels at which maximum yields were observed for each species are presented in Table 6.3.

Examining the yields of the six clover species across individual harvest dates showed that *T. glomeratum* and *T. subterraneum* responded positively to S addition during the early growth stages (harvests 10, 13 and 17 weeks post germination respectively), with *T. arvense* showing yield responses to S at harvests 13, 17, 23 and 29 weeks post germination respectively. *Trifolium striatum* and *T. dubium* showed a yield response to added soil S during later growth stages (23 weeks post germination).
Figure 6.1 Total accumulated shoot dry matter (TDM) yield response of clover species: A, *T. glomeratum*; B, *T. arvense*; C, *T. subterraneum*; D, *T. dubium*; E, *T. striatum*; F, *T. repens* to increasing levels of soil sulphur (7 levels of S; ranging from 0–240 mg S/kg soil), grown in a South Island high country soil. Data are mean values ± SEM (*n*=4), with *P* and *R*² values for fitted curves showing data trend.
Table 6.2  Values of shoot yield, shoot S concentration, and shoot S uptake by six clover species, grown under glasshouse conditions in a NZ high country soil supplied with increasing rates of S; S application (7 levels of S; ranging from 0–240 mg S/kg soil).

<table>
<thead>
<tr>
<th>Species</th>
<th>Shoot Yield (g DM/pot)</th>
<th>Mean Shoot S Concentration (%)</th>
<th>S Uptake (mg S/pot)</th>
<th>Soil SO₄⁻-S¹ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. glomeratum</td>
<td>4.78</td>
<td>0.20</td>
<td>9.3</td>
<td>16</td>
</tr>
<tr>
<td>T. arvense</td>
<td>6.98</td>
<td>0.18</td>
<td>11.6</td>
<td>16</td>
</tr>
<tr>
<td>T. subterraneum</td>
<td>7.71</td>
<td>0.21</td>
<td>15.7</td>
<td>13</td>
</tr>
<tr>
<td>T. dubium</td>
<td>5.77</td>
<td>0.23</td>
<td>11.5</td>
<td>14</td>
</tr>
<tr>
<td>T. striatum</td>
<td>4.86</td>
<td>0.24</td>
<td>10.9</td>
<td>18</td>
</tr>
<tr>
<td>T. repens</td>
<td>4.57</td>
<td>0.17</td>
<td>7.7</td>
<td>16</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>5.78</td>
<td>0.21</td>
<td>11.1</td>
<td>16</td>
</tr>
<tr>
<td>Species</td>
<td>LSD (5%)</td>
<td>0.317</td>
<td>0.010</td>
<td>0.600</td>
</tr>
<tr>
<td>S Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg S/kg soil)</td>
<td>0</td>
<td>5.64</td>
<td>0.15</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.84</td>
<td>0.16</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>6.04</td>
<td>0.17</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.48</td>
<td>0.20</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.94</td>
<td>0.21</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.82</td>
<td>0.25</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>5.68</td>
<td>0.31</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>LSD (5%)</td>
<td>0.342</td>
<td>0.010</td>
<td>0.65</td>
</tr>
</tbody>
</table>

\( P \)

<table>
<thead>
<tr>
<th>Species</th>
<th>***</th>
<th>***</th>
<th>***</th>
<th>**</th>
</tr>
</thead>
<tbody>
<tr>
<td>S Rate</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sp x S Rate</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

¹ Soil Sulphate S content at the completion of the experiment ***, **, * indicate the treatment effect being significant at \( P<0.001, P<0.01, P<0.05 \) respectively.
Table 6.3  Rate of S application and shoot S concentration at which maximum yield was observed for each clover species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum yield (g DM/pot)</th>
<th>S rate (mg S/kg soil)</th>
<th>Shoot S concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. glomeratum</td>
<td>5.3</td>
<td>10</td>
<td>0.17</td>
</tr>
<tr>
<td>T. arvense</td>
<td>7.4</td>
<td>20</td>
<td>0.15</td>
</tr>
<tr>
<td>T. subterraneum</td>
<td>8.0</td>
<td>120</td>
<td>0.27</td>
</tr>
<tr>
<td>T. dubium</td>
<td>6.2</td>
<td>20</td>
<td>0.15</td>
</tr>
<tr>
<td>T. striatum</td>
<td>6.2</td>
<td>20</td>
<td>0.15</td>
</tr>
<tr>
<td>T. repens</td>
<td>5.4</td>
<td>60</td>
<td>0.25</td>
</tr>
</tbody>
</table>

6.4.2 S concentration and uptake

All clover species showed a trend of increasing shoot S concentrations with increasing S application rate (Table 6.2 and Figure 6.2). Shoot S concentration in T. subterraneum cv. Mt Barker and T. dubium showed a steady increase, ranging from 0.12–0.39 and 0.15–0.38% S respectively. Sulphur in the shoots of T. glomeratum (0.14–0.27% S), T. arvense (0.12–0.25% S) and T. striatum (0.17–0.35% S) showed more curvilinear increases in concentration in response to increasing rates of applied S. The only perennial clover species in the group, T. repens cv. Nomad, showed minimal increase in shoot S concentration as soil S supply increased, with a range of 0.15–0.19% S across all S fertiliser treatments. The order of greatest mean shoot S concentration was as follows: T. striatum > T. dubium > T. subterraneum > T. glomeratum > T. arvense > T. repens (Table 6.2). There was no discernible relationship between total accumulated DM yield and shoot S concentration in any of the clover species. This is further indication that these species were relatively unresponsive to added S. The pattern of S uptake by the clover species showed to be similar in nature to that of S concentration (Table 6.2).
Figure 6.2 Comparison of shoot S concentration of clover species: A, *T. glomeratum*; B, *T. arvense*; C, *T. subterraneum*; D, *T. dubium*; E, *T. striatum*; F, *T. repens* to increasing levels of soil sulphur (7 levels of S; ranging from 0–240 mg S/kg soil), grown in a South Island high country soil. Data are mean values ± SEM (n=4), with *P* and *R*² values for fitted curves showing data trend.
6.4.3 Lime yield response

Lime addition significantly influenced the yield of all clover species \( (P<0.001, \text{Table 6.4}) \), though this effect differed between species (Figure 6.3). Mean TDM yields ranged from 3.6–6.5 g DM/pot by trial end (Table 6.4). *Trifolium arvense* was the most productive clover species with a maximum TDM yield of 9.2 g DM/pot, produced at the most acidic soil condition (pH 5.2; lime rate 0 t/ha), followed by *T. subterraneum* (8.6 g DM/pot) and *T. striatum* (8.0 g DM/pot) (Figure 6.3). The lowest yielding species was *T. repens* cv. Nomad (4.3 g DM/pot). *Trifolium repens* cv. Nomad and *T. striatum* were the only clover species to show a significant positive yield response to increasing soil pH levels above 5.2, as a result of lime addition (up to 2 t lime/ha) (Figure 6.3). All other clover species were tolerant of more acidic soil pH levels as maximum TDM yields were produced at low pH (pH 5.2–5.7) (Figure 6.3). At the higher liming rates of 4 and 8 t/ha equivalent, all species showed a significant reduction in yield response (Figure 6.3), producing poor TDM yields at higher pH levels of 6.3 and 7.2 respectively. All species showed a general decline in shoot P concentration as soil pH level increased towards 7.2 resulting from increased lime addition (Figure 6.4). In contrast, all species showed a strong increase in molybdenum (Mo) concentration in shoots as soil pH level increased in response to increased lime addition (Table 6.4).
Table 6.4  Values of shoot yield, shoot S concentration, and shoot S uptake by six clover species, grown under glasshouse conditions in a NZ high country soil supplied with increasing rates of lime application (5 rates of CaCO₃; ranging from 0–8 t/ha equivalent).

<table>
<thead>
<tr>
<th>Species</th>
<th>Shoot yield (g DM/pot)</th>
<th>Mean shoot P Concentration (%)</th>
<th>Mean shoot Mo Concentration (mg/kg)</th>
<th>Soil pH¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. glomeratum</td>
<td>4.14</td>
<td>0.24</td>
<td>0.52</td>
<td>6.0</td>
</tr>
<tr>
<td>T. arvense</td>
<td>6.48</td>
<td>0.24</td>
<td>1.45</td>
<td>5.9</td>
</tr>
<tr>
<td>T. subterraneum</td>
<td>6.20</td>
<td>0.23</td>
<td>0.54</td>
<td>6.0</td>
</tr>
<tr>
<td>T. dubium</td>
<td>4.72</td>
<td>0.30</td>
<td>0.89</td>
<td>6.1</td>
</tr>
<tr>
<td>T. striatum</td>
<td>4.66</td>
<td>0.24</td>
<td>0.50</td>
<td>6.0</td>
</tr>
<tr>
<td>T. repens</td>
<td>3.56</td>
<td>0.23</td>
<td>0.56</td>
<td>5.9</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>4.96</td>
<td>0.25</td>
<td>0.74</td>
<td>6.0</td>
</tr>
<tr>
<td>Species</td>
<td>LSD (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. glomeratum</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. arvense</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. subterraneum</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. dubium</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. striatum</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. repens</td>
<td>2.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Mean</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime Rate</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime Rate</td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime Rate</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime Rate</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime Rate</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Soil pH at the completion of the experiment ***, * indicate the treatment effect being significant at P<0.001, P<0.05 respectively.
Figure 6.3 Total accumulated shoot dry matter (TDM) yield response of clover species: A, *T. glomeratum*; B, *T. arvense*; C, *T. subterraneum*; D, *T. dubium*; E, *T. striatum*; F, *T. repens* to increasing levels of lime (5 levels of Lime; ranging from 0–8 t/ha equivalent), grown in a South Island high country soil. Data are mean values ± SEM (n=4), with P and $R^2$ values for fitted curve showing data trend.
Figure 6.4 Comparison of shoot P concentration (%) of clover species: A, *T. glomeratum*; B, *T. arvense*; C, *T. subterraneum*; D, *T. dubium*; E, *T. striatum*; F, *T. repens* to increasing levels of lime (5 levels of Lime; ranging from 0–8 t/ha equivalent), grown in a South Island high country soil. Data are mean values ± SEM (n=4), with $P$ and $R^2$ values for fitted curve showing data trend.
6.5 Discussion

6.5.1 S response

Under these conditions of low initial soil SO$_4^{2-}$-S status, most of the clover species were either responsive at low S application rates, or were unresponsive to S addition (e.g. T. repens cv. Nomad; Figure 6.1). The recommended soil SO$_4^{2-}$-S level range for near-maximum pasture production on sheep and beef farms in New Zealand is 10–12 mg S/kg (Morton & Roberts 2004), therefore a growth response to added S was expected in this experiment as the soil used had a low SO$_4^{2-}$-S level of 5 mg S/kg (Table 6.1). Yield responses in the order of 12–17% were observed for those species which were responsive to S (T. arvense, T. dubium and T. striatum).

Smith et al. (2004) reported a lack of response to S application by T. subterraneum plants growing in summer hill and high country despite very low soil SO$_4^{2-}$-S levels for the control plots (<3 mg S/g). They suggested T. subterraneum is tolerant of low soil S supply. The results of this current study strongly support this finding, as T. subterraneum was generally unresponsive to S addition (Figure 6.1). Conversely, the yield response of T. repens in this glasshouse experiment is in disagreement with previous work, which though conducted under field conditions, showed T. repens to be more responsive to S than P (Sinclair et al. 1996; Morton et al. 1998). A possible explanation is that mineralisation of S (and C) occurred during the course of this glasshouse experiment causing a ‘flush’ of readily plant-available S, as reported by Tsuji & Goh (1979). However, post-experiment soil tests showed indicated that target SO$_4^{2-}$-S levels for the intended S treatment levels were achieved (Table 6.2), indicating mineralisation as an unlikely explanation. This is supported by the fact that some of the species were indeed S responsive. Boswell et al. (2007) reported that T. arvense had DM (and large N fixation) responses to S, which is agreement with the results of this study.

Morton et al. (1999a) found pasture herbage (grass and legume) S concentration increased from 0.25 to 0.4% S as the application rate of S increased from 0 to 80 kg/ha and the overall pattern of the current results is consistent with these values. Craighead & Metherell (2006) suggested an optimum herbage level of 0.18–0.22% S for NZ hill and high country areas, with deficiency below 0.15% S. The data from the current work indicate that the clovers had low % S in the control treatments, which increased with increasing S rate, for all species. However, there was no corresponding yield response for T. glomeratum, T. subterraneum and T. repens, indicating that factors other than soil SO$_4^{2-}$-S levels were limiting yield and that
luxury S uptake occurred where S was applied. It is therefore possible that these three species have a low S requirement for optimum growth. Maxwell et al. (2010) found no correlation between the abundance (percentage vegetative cover) of these naturalised annual clover species and soil SO$_4$-S level in a survey of two South Island hill/high country farms, in which edaphic influence (soil pH and available P and S) on these species was investigated; which is consistent with the results of this experiment. The current results suggest that the Nomad cultivar of *T. repens* may have lower S requirements for optimum growth than the other clover species investigated in this experiment. Further glasshouse research is required to confirm this result, before warranting field research to follow.

The concentration of P in the shoots of *T. repens* cv. Nomad, across all S rates, was at optimum (>0.25% P; McLaren & Cameron 2005); therefore we can discount that P was deficient and thus restricting the growth of *T. repens* cv. Nomad. From herbage analyses, other macro and micro nutrients were also non-limiting (McLaren & Cameron 2005). *Trifolium repens* cv. Nomad’s lack of yield response may be explained by the very small increases in shoot S concentration, even with very large inputs of S fertiliser. This is an unusual result however for a clover species considered to be high yielding with moderate to high fertiliser requirements (Caradus et al. 1995). It was the only species to behave in this manner. Peak yield was 5.1 g DM/pot, which was higher than that observed in another experiment (T.M.R. Maxwell; Chapter 5) where yield response by the same six species to added P was investigated. This fact alone suggests that S was not limiting, however the S yield response of *T. repens* in this experiment contrasts with previous work.

### 6.5.2 Lime response

At a base soil pH level of 5.2, the literature suggests that lime addition reduces exchangeable (plant available) Al levels (Moir & Moot 2010) while increasing P and Mo availability in the soil and/or increasing the plants ability to use available P (Wheeler & O’Conner 1998). All these factors, acting either individually or collectively, would be expected to result in a general positive yield response to lime addition to soil within a pH level range of 5.2–6.0. Interestingly however, in this study, only two species (*T. repens* and *T. striatum*) showed a positive yield response to lime addition and no further response at higher lime rates, while all other species showed negative yield responses to lime addition, above 2 t/ha equivalent (Figure 6.3). All adventive clover species and *T. subterraneum* cv. Mt Barker showed highest TDM yields between the base soil pH 5.2 and pH 5.48 (Figure 6.3). These data therefore
provide strong new evidence that these annual clover species are tolerant of acid soil conditions. No relationship between soil pH and herbage S concentration was observed.

Cordero & Blair (1978) reported *T. subterraneum* performed equally well at pH 5.9, and under the more acidic condition of pH 4.4 in a glasshouse experiment. Cordero & Blair (1978) found *T. glomeratum* was the most responsive to lime addition to a soil of an initial pH of 5.0, though yielding only half that of *T. subterraneum* and *Ornithopus compressus*. Edmeades et al. (1991) found *T. subterraneum* was not sensitive to low pH, though moderately sensitive to Al. Hayes et al. (2008) observed *T. subterraneum* to be more tolerant of acid soil conditions (pH 5.3) than *T. michelianum* Savi and *T. glanduliferum* Boiss, with no visible symptoms of manganese toxicity and no response to lime (2.9 t/ha) in terms of seed yield. These results are in accord with the results of this glasshouse study, though *T. glomeratum*, like *T. subterraneum*, did not show a positive yield response to lime addition.

All species exhibited a negative yield response at high lime rates (>2 t/ha equivalent) where soil pH level rose above 5.7 (Table 6.4 and Figure 6.3). This can be attributed to the influence of greater soil pH levels on the availability of P, and trace elements (Mo, B and Cu) brought about by increasing rates of lime addition. Figure 6.4 and Table 6.4 show that shoot P concentration generally decreased as pH increased from 5.2 to 7.2. Though annual clover lime response trails under glasshouse conditions in NZ are scarce, a field trial by Lambert & Grant (1980) found that southern North Island hill country pasture legume vigour and soil plant-available P showed positive responses to low lime rate application (0–3.5 t/ha), with a small negative response in legume vigour at higher lime rates. This was attributed to the depression of P availability at high pH (>6.0) due to calcium phosphate formation (Larsen et al. 1965), which may have been the reason for yield depression at higher liming rates in the current experiment.

Lambert and Grant (1980) found that legume Mo concentration increased in response to lime addition, which is in agreement with the findings here (Table 6.4). Herbage analysis showed that Mo was high (5.0 mg/kg) while boron (2.0 mg/kg) and copper (3.0 mg/kg) were deficient (Sherrell 1983; Morton et al. 1999b) in the shoots of clovers grown at pH 7.2 (8 t lime/ha equivalent). Therefore, in addition to a clear liming/high pH-induced P deficiency, conditions of B and Cu deficiency were induced at the highest lime rate of 8 t/ha equivalent in this experiment. At 3–4 weeks post germination, seedlings of all clover species growing in the 8 t/ha lime treatment showed poorer seedling emergence with darker green foliage of rolled and
less-erect appearance. It is possible that these seedlings were also inhibited by high pH-induced trace element deficiencies (B and Cu). It does not appear that any Mo toxicity was induced in our experiment, as the highest shoot Mo concentrations were well below the level at which toxicity effects on plants has been observed (<500 mg/kg) (Gupta 1997).

6.5.3 Implications for low input extensive grazing systems

These naturalised, adventive annual clover species appear to have the ability to grow well in a soil with a low pH, low natural S and high exchangeable Al levels under glasshouse conditions. Maxwell et al. (2010) found that soil pH and SO₄²⁻-S did not significantly influence the abundance of these naturalised annual species or _T. repens_ in the field. The results of this experiment support the findings of Maxwell et al. (2010) as these species appeared to be generally low or unresponsive to S, and most species were able to grow well in acidic soil conditions. Soil S appeared to be sufficient for optimum growth by the clover species in this glasshouse experiment, even at a low initial soil SO₄²⁻-S. It should be noted that the annual clovers used here were ecotypes and may not be representative of the species as a whole.

Field S response experiments are required to confirm the results of this experiment. Possibly, field S fertility and fertiliser requirements may be less than previously expected for some hill and high country soils in New Zealand, in order to maintain adequate S fertility for this suite of adventive clover species that dominate in these dry hill and high country environments (Maxwell et al. 2010).

Boswell et al. (2003b; 2007) reported large increases in N fixation by _T. arvense_ in semi-arid (<600 mm) high country in response to added S fertilisers in wetter than average growing seasons. Though small increases in DM may occur in response to added S, the relationship between increased S fertility and greater N inputs from resident annual clover species is an important avenue for further investigation in hill and high country pasture improvement in summer dry areas.

Optimum pH for pasture plant growth has been defined as the pH level above which no further response to lime occurs (Edmeades et al. 1984). The results of this experiment would suggest that optimum pH ‘range’ for these six clover species is between pH 5.2–5.7 (Table 6.4 and Figure 6.3). Relatively low rates of lime application (1 t/ha) can produce worthwhile
pasture responses on south-facing (shady) aspects and easy slopes, which favour legume growth in dry hill country (Morton et al. 2005).

6.6 Conclusions

Annual clover species had low response or were unresponsive to added S. The perennial *Trifolium repens* cv. Nomad was unresponsive to added S. Naturalised, adventive annual clover species, *T. subterraneum* cv. Mt Barker and *T. repens* cv. Nomad produced comparably high TDM yields at low levels of SO₄²⁻–S, adding value to the contention that available P level is the key factor for clover performance in the context of hill and high country soil fertility. Under the climatic, soil basal fertility and moisture conditions created in this glasshouse experiment, these species appear to have low S requirements for near optimum production in terms of shoot S concentration and S uptake.

*Trifolium striatum* and *T. repens* cv. Nomad were the only clover species to show a positive yield response to increasing soil pH levels above the natural acidic level of 5.2. All other species had negative yield responses to lime addition. Negative yield responses were due to a high soil pH-induced reduction in the availability of P, B and Cu. *Trifolium arvense*, *T. subterraneum* cv. Mt Barker, *T. dubium* and *T. glomeratum* were tolerant of acidic soil pH levels and produced highest TDM yields from pH 5.2–5.5. All adventive annual species and *T. subterraneum* cv. Mt Barker produced greater yields than *T. repens* cv. Nomad.

These annual clovers warrant further investigation under field conditions. The presence and spread of these adventive annual clover species in acid, summer dry, high and hill country environments may prove beneficial where traditionally sown clovers fail to persist.

Acknowledgements

This research was funded by the Miss E L Hellaby Indigenous Grasslands Research Trust. We thank Chas Todhunter of Glenfalloch Station, Dave Jack, Daniel Dash, Francis Malcolm, Carole Barlow, Angela Reid, Lynne Clucas, and Fiona McConville of Lincoln University, and Sho Kasuya, Hugh Jackson, Kylie Wright, Matt Dalziel, Philip Jones and Dr Yoshitaka Uchida for technical assistance.
Chapter 7
Grazing preference of Merino sheep for adventive annual clover species relative to sown clover species
Trifolium repens and Trifolium subterraneum

7.1 Introduction
Grazing behavioural choices of livestock, manifested as either grazing preference or selection for different pasture species and plant components, play an influential role in the steady state botanical composition of grazed pasture swards (Cosgrove et al. 1999; Harvey et al. 2000). Grazing preference is defined as what animals select in an environment of minimal physical constraints (Parsons et al. 1994) whereas selection is defined as animal preference modified by environmental circumstances (Hodgson 1979). In extensive high and hill country grazing environments, knowledge of the different resident clover species, specifically their grazing preference and diet selection by sheep, aids efficient livestock production (Cosgrove et al. 1999). An understanding of herbivore foraging decisions in response to pasture sward state and the relative availability/abundance of plant species (resident or potentially resident following introduction) is required for further development of sustainable grazing systems (Harvey et al. 2000). Such knowledge may help improve feed intake (animal performance/production) via manipulation of grazing preference and selection (Edwards et al. 2008).

Literature exists on grazing preference and selection of white clover (Trifolium repens L.) and perennial ryegrass (Lolium perenne L.) by sheep and cattle in temperate pastures. Results show that domesticated ruminants select a mixed diet, showing about 70% partial preference for white clover over ryegrass when offered as monoculture swards (Rutter 2010). However, literature on grazing preference by sheep, specifically Merino types, for less well researched annual clover species, naturalised and adventive to New Zealand grazed agro-ecosystems within dry regions (<800 mm annual rainfall), is scarce or does not exist. Such naturalised, volunteer annual clover species are commonly found in lower-fertility, extensively-grazed pasture systems in summer dry and semi-arid regions of New Zealand (Boswell et al. 2003; Power et al. 2006; Maxwell et al. 2010). These include cluster clover (Trifolium glomeratum L.), suckling clover (T. dubium Sibth), haresfoot clover (T. arvense L.), and striated clover (T. striatum L.).
Diet selection by domesticated ruminants can affect pasture community composition (Cosgrove & Edwards 2007), whereby selective grazing of preferred species (*Trifolium subterraneum* and *Lolium rigidum*) can give more unpreferred, weed species (*Arctotheca* sp. and *Erodium* sp.) a competitive advantage (Broom & Arnold 1986). Colebrook et al. (1990) stated changes in preference for a pasture species over the growing season can influence the persistence of a species; a species less preferred (less preferentially grazed) during early stages of growth would be assisted in its establishment in a grazed sward, in contrast to being more preferred during reproductive stages (flowering and seed set) which would act against its persistence in the sward.

Australian research has found that Merino sheep adopt different foraging strategies in response to changes in annual clover herbage characteristics. Forage chemical characteristics appear to influence sheep preference among annual legumes more at reproductive and senesced stages of maturity, rather than at the vegetative stage. Preference for a particular pasture/forage legume species is greater if the animal can increase intake of digestible dry matter as the annual legume sward matures (Thomas et al. 2010).

The objective of this chapter is to report on the investigation of Merino sheep grazing preference for four adventive annual clover species naturalised in New Zealand and common in summer dry hill and high country areas, compared to two commonly sown clovers as reference species (*T. repens* and *T. subterraneum* L.), as a basis to understanding changes in plant species composition; with the hypothesis that grazing preference for clovers differs between species, and changes as plants become reproductive.

### 7.2 Methodology

#### 7.2.1 Field location, preparation & experimental design

Two grazing preference tests were carried out in a 0.06 ha field area within research block H18, a flat paddock of long pastoral and horticultural research history at Lincoln University. The soil type was a Wakanui silt loam with moderate to high soil fertility (Table 7.1). The experiment was designed as a randomised complete block design, with a 30 x 5 m area containing seven 2 x 1 m different clover species swards within a mixed-perennial grass background, predominately comprised of browntop (*Agrostis capillaris* L.) and perennial ryegrass (*Lolium perenne* L.), replicated four times (Plate 7.1). Individual clover swards were
monocultures of each clover species and one mixed stand comprising all 6 species in combination. Each clover sward was spatially separated by 2 m of mixed-perennial grass.

Table 7.1 Soil chemical properties of 0.06 ha field experiment area used for grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, November (spring) 2009.

<table>
<thead>
<tr>
<th>Soil chemical properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.1</td>
</tr>
<tr>
<td>Olsen P</td>
<td>39 ug/mL (mg/L)</td>
</tr>
<tr>
<td>Sulphate-S</td>
<td>10 ug/g (mg/kg)</td>
</tr>
<tr>
<td>Organic-S</td>
<td>3 ug/g (mg/kg)</td>
</tr>
<tr>
<td>Exchangeable K</td>
<td>26 QTU (cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Mg</td>
<td>34 QTU (cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>7 QTU (cmol/kg)</td>
</tr>
<tr>
<td>Exchangeable Na</td>
<td>9 QTU (cmol/kg)</td>
</tr>
</tbody>
</table>

Prior to the pure clover swards being sown in April 2009, resident pasture in H18 was sprayed out in March with a glyphosate-based broad-spectrum herbicide, Roundup Transorb, at a rate of 2 L/ha. Subsequent standing dead pasture cover within marked out 2 x 1 m plots for clover swards was removed by mowing plots to ground level to expose bare ground, followed by gentle cultivation of the soil surface using a garden rake.

7.2.2 Clover sward establishment

Seeds of the respective clover species were gently scarified with sand paper to breakdown hard seed coats and increase seed germination. Scarified seeds were then mixed with fine sand for broadcasting at a 500 seeds/m² rate. Prior to broadcasting, plot areas were watered to moisten the soil surface layer, with the respective clover seed and sand mix then broadcast evenly over each 2 x 1 m plot area. After broadcasting of seed, the soil surface of each clover plot was lightly raked in both a long ways and sideways direction to evenly cover and bury broadcasted seed, returning any seed and soil that had stuck to the fingers of the rake to the plot. Plots were watered daily for one week to promote germination and seedling emergence and ensure maximum seedling survival. After establishment, plots were managed for 6
months to achieve abundant and dense pure clover swards by hand weeding of any volunteer herb, clover or grass species, and watering during dry periods.

Plate 7.1  Experimental layout for grazing preference tests, prior to erection of electric flexi-netting. Plots of 2 x 1 m dimension are pure swards of each clover species within a perennial grass background, at H18 Lincoln University, Canterbury, New Zealand, November 5th 2009.

7.2.3 Field Measurements

Two grazing events (preference tests) with sheep were conducted during late spring (10th November) and early summer (16th December) of 2009; 30 weeks and 35 weeks post sowing respectively. Prior to releasing sheep onto grazing preference test blocks (strips), pre-graze clover sward measurements were made of sward surface height (SSH) and clover sward herbage biomass (dry matter yield; DMY).

Sward surface height was measured using a sward stick, with 20 height measurements taken from each clover sward within each block (Plate 7.2) and mean pre-graze SSH calculated. Sward biomass of each clover plot was measured by cutting all herbage present within paired 0.2 m² quadrats to ground level. The first cut was done before grazing and the second cut
done after grazing, with the location choice of the two-quadrat cuts being made where herbage of similar height and density was observed, prior to grazing. Post-graze SSH and clover mass measurements were collected in the same fashion as pre-graze measurements.

Sward morphology was determined by sorting a subsample of the pre-graze and post-graze herbage from quadrat cuts into leaf, stem, petiole, flower, dead, and other (non-clover) components, then drying at 70ºC for 48 hours and weighing.

Plate 7.2  Sward stick used for measuring clover sward height (cm), Lincoln University, Canterbury, New Zealand, November 5th 2009.

7.2.4 Relative Preference

Clover sward intake was estimated from the clover sward DM available before grazing minus the DM remaining post grazing (Thomas et al. 2010). Grazing preference is determined in relation to other forage options available to an animal grazing under minimal environmental constraints (Newman et al. 1995). Preference here therefore is expressed relative to all other available clover swards on offer. Relative preference among the clover species swards was thus calculated from initial clover sward biomass and estimated clover sward intake using the
Chesson-Manly selection index (Manly et al. 1972; Chesson 1983) as used by Smit et al. (2006) and Thomas et al. (2010). The Chesson-Manly selection index is a methodology that accounts for forage availability and depletion with the assumption that the probability of a grazing animal encountering a preferred plant decreases more rapidly than for plants that are less preferred (Smit et al. 2006).

7.2.5 Chemical analysis

Pre-graze clover herbage samples were halved, with one portion being freeze-dried for subsequent nutritive analysis. Herbage nutritive analysis was carried out via near-infrared spectroscopy (NIRS) to determine metabolisable energy (MJ ME/kg DM) and percentage concentrations of acid detergent fibre (ADF), neutral detergent fibre (NDF), protein (CP), dry matter digestibility (DMD), organic matter (OM), and water soluble carbohydrate (WSC) of each clover species collected in late spring and early summer.

7.2.6 Animals and behaviour measurements

The breed and sex of sheep used for the grazing preference tests were twelve Merino Dorset crossbred ewe hoggets selected from a flock at Lincoln University’s dryland sheep farm, Ashley Dene. In addition, four 5 year old Merino wethers (castrate males) were selected from Lincoln University’s Field Service Centre sheep flock. Sheep were drafted into four groups, made up of one wether and three ewe hoggets respectively. Sheep in each replicate were colour branded on the head and rump to allow quick identification during grazing behaviour observations (Plate 7.3). The behaviour of every sheep in each block was recorded at 5-minute intervals for the duration of the two grazing preference tests. Each individual sheep were scored based on which clover plots they were grazing from, on whether they were grazing grass, or showing non-grazing behaviour (walking, idling, or ruminating). Prior to grazing preference test commencement, all sheep had free access to abundant pasture cover containing various perennial grass species and clover species, adjacent to the grazing preference test area.
Plate 7.3  Merino Dorset cross sheep with colour branding on rump (and head) for the purpose of quick identification when recording grazing behaviour during grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, November 10th 2009. NB Sheep are grazing from a 2 x 1 m clover sward area.

The perennial grass background was mown to 10 cm height before releasing the sheep onto grazing preference strips. Temporary fencing (flexi-netting) was used to demarcate each replicate (block) of the six clover species swards and one mixed-species sward (Plate 7.4). Sheep were released onto clover plots at 11.30am on November 10th, and at 3.10 pm on 16th December 2009. Grazing behaviour observation lasted for four hours. Grazing observation was carried out from a platform on a viewing tower, elevated 7 metres above ground, next to the grazing preference test area (Plate 7.5).
Plate 7.4  Flexi-netting used for separating grazing blocks (replicates) and four groups of Merino Dorset cross sheep during grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, November 10th 2009.

7.2.7 Statistical Analysis

One-way ANOVA was conducted on mean SSH, clover biomass, SSH reduction, sward offtake and utilisation, relative preference, grazing time, and clover herbage chemical composition data collected on both preference test dates (Nov 10th and Dec 16th) with different clover species swards as the primary factor. One way ANOVA was conducted on mean leaf, petiole, stem, flower, dead and other (non-clover) sward morphological component data for each clover species collected on both preference test dates. Correlation coefficients were generated between relative preference, and the structural (pre-graze SSH, pre-graze sward biomass) and chemical (ADF, NDF, WSC, CP, OM, DMD, ME) components of each clover species, in order to identify any significant correlations between relative preference and sward components. Linear regressions models were generated from significant correlations between relative preference and structural or chemical components. Data was analysed using GenStat 14 statistical software package (Lawes Agricultural Trust, Rothamsted, UK).
Plate 7.5  Viewing tower with observation platform from which grazing behaviour observations were made during grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, mid November 2009.

7.3 Results

Mean sward surface height (SSH) and mean sward biomass (kg DM/ha), before and after grazing, mean changes in sward height and biomass, and the Chesson-Manly relative preference index of each clover sward are presented in Table 7.2. Data represent two different phenological stages/stages of plant maturity; predominately vegetative, in mid-late spring (November 10th) and predominately reproductive, in early summer (16th December).
7.3.1 Sward surface height

Mean SSH ranged from 6.3–14.6 cm prior to grazing on November 10th, and from 5.1–11.2 cm prior to grazing on December 16th. The order of clover SSH prior to the Nov 10th grazing preference test was T. dubium > T. glomeratum > T. subterraneum > T. striatum > mixed species sward > T. arvense > T. repens. This order was different prior to the Dec 10th grazing preference test with T. repens > T. arvense > T. dubium > T. glomeratum > mixed species sward > T. subterraneum > T. striatum. Clover species pre-graze SSH were not significantly different from each other prior to grazing preference tests on Nov 10th or Dec 16th (Table 7.2).

Sheep grazing significantly reduced mean SSH (%) during the November (P<0.01) and December (P<0.001) preference tests, ranging from 24–58% and from -3.3–63.2% respectively. Trifolium subterraneum showed the greatest reduction (58%) during the November test followed by T. striatum, T. arvense (both 50%), T. glomeratum (47%) and T. repens (42%), with mixed clover species and T. dubium swards showing least reduction in height at 38% and 24% respectively (Table 7.2).

During the second grazing preference test in December, Trifolium repens swards were reduced by 63% in comparison to 42% in November, while SSH reduction of Trifolium arvense (47%) and the mixed clover species sward (41%) did not change greatly. Trifolium subterraneum and T. glomeratum swards dropped 46% and 31% in height respectively which was less than in November. Trifolium striatum swards showed a much lower magnitude in SSH reduction at only 12% compared to Nov 10th while T. dubium swards showed minimal change (Table 7.2).

Figure 7.1a shows the relationship between pre-graze SSH and SSH reduction from grazing between the two sampling dates. There was a strong positive correlation between the initial pre-grazing SSH and the magnitude of SSH reduction (R² = 0.80) with more height reduction observed from initially taller swards on Nov 10th. However, the relationship between pre-graze SSH and SSH reduction was very weak (R² = 0.19) 5 weeks later on Dec 16th.
7.3.2 Clover sward biomass

Clover sward mass ranged from 753–2589 kg DM/ha prior to grazing on Nov 10\textsuperscript{th}, and from 904–2684 kg DM/ha prior to grazing on Dec 16\textsuperscript{th}. There were significant differences ($P<0.001$) in biomass among the clover swards in November with $T.\ repens$ the lowest yielding species at 753 kg DM/ha. Three swards ($T.\ arvense$, mixed species, and $T.\ glomeratum$) yielded intermediate levels of biomass at 1107, 1290, and 1893 kg DM/ha respectively. $Trifolium\ striatum$, $T.\ dubium$ and $T.\ subterraneum$ yielded highest biomass levels at 2509, 2543, and 2589 kg DM/ha respectively. Significant differences in biomass among the clover swards were also evident in December ($P<0.01$) with $T.\ arvense$ producing the least biomass at 904 kg DM/ha, followed by $T.\ repens$ (1203 kg DM/ha) and $T.\ dubium$ (1617 kg DM/ha). Mixed species (1290 kg DM/ha) and $T.\ glomeratum$ (1994 kg DM/ha) yielded intermediate levels with $T.\ subterraneum$ (2006 kg DM/ha) and $T.\ striatum$ (2684 kg DM/ha) producing the greatest herbage biomass (Table 7.2).

Figure 7.1b shows a positive relationship between the level of clover sward biomass and the amount of offtake from grazing between the two sampling dates. Clover offtake was strongly associated with initial pre-graze clover sward mass on offer. Greater clover offtake occurred from swards of greater initial biomass. The curvilinear relationship between initial clover sward mass and subsequent offtake was strong in both November ($R^2 = 0.67$) and December ($R^2 = 0.58$).
Table 7.2  Mean pre-graze and post-graze sward surface height (SSH, in cm), pre-graze and post-graze sward mass (kg DM/ha), clover offtake, Chesson-Manly relative preference index, and grazing time (mins) of the six clover species and mixed clover species plots at two dates in mid-spring (10th November) and early summer (16th December) prior to grazing preference tests at field H18, Lincoln University, Canterbury, New Zealand, 2009. NB *** , ** and * indicate the component being significantly different at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ respectively, ns = not significant. Different letters denote means that are significantly different @ 5% level.

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<th>Suckling</th>
<th>White</th>
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<th>SEM</th>
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<td>2589 c</td>
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<td>931 bc</td>
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<td>0.139</td>
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<td>1772 bc</td>
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<td>2006 cd</td>
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<td>1710</td>
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<td>751 bc</td>
<td>1069 c</td>
<td>845 bc</td>
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<td>1021 bc</td>
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<td>1161 cd</td>
<td>552 ab</td>
<td>942 abc</td>
<td>960</td>
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<td>59 bc</td>
<td>59.8 bc</td>
<td>60.6 bc</td>
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<td>0.035*</td>
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<td>0.141 a</td>
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<td>0.248 b</td>
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<td>7.5 a</td>
<td>13.8 bc</td>
<td>17.8 c</td>
<td>23.8 d</td>
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<td>11.6 b</td>
<td>10 b</td>
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<td>15.3 b</td>
<td>3.1 a</td>
<td>16.3 b</td>
<td>11.6</td>
<td>2.3</td>
<td>0.01**</td>
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Figure 7.1 Relationship between (a) sward surface height (SSH) and reduction in SSH (cm), and (b) pre-graze clover sward biomass and clover offtake by Merino sheep.
Clover sward utilisation (%) varied from 46–77.5% on Nov 10th and from 33–77% on Dec 16th. During both preference tests *T. dubium* was the least grazed clover sward, while *T. repens* was the most grazed. The % utilisation between clover swards was significantly different (*P*<0.05, Table 7.2) on Dec 16th when all species were predominately reproductive but not on Nov 10th when all clover swards, other than *T. dubium*, were mostly vegetative. *Trifolium dubium* is the earliest to flower among the six clover species sown in this experiment and thus the most rapid maturing species.

From the November to December preference tests, utilisation (%) varied more for clover swards of *T. glomeratum*, *T. arvense* and *T. dubium* while the mixed species sward, *T. striatum*, *T. subterraneum* and *T. repens* remained either the same or changed minimally. *Trifolium glomeratum* swards were more utilised in November (74.5%) than December (45%). *Trifolium arvense* was also more utilised in November (72%) than December (59%). *Trifolium dubium* also showed this trend of decreasing utilisation, but was already the least utilised clover species in November at 46%, which reduced further to 33% in December. The moderately high utilisation of *T. subterraneum* (<60%) was consistent from the November test to the December test. Similarly to *T. repens*, utilisation of *T. striatum* and the mixed species sward varied the least out of all the clover swards at about 60%. The mixed species sward also showed moderately high utilisation at 59% in both November and December. *Trifolium repens* utilisation was consistently very high (>75%) in both November and December (Table 7.2).

### 7.3.3 Relative preference

Relative preference varied between 0.077 for *T. dubium* (least preferred) and 0.191–0.192 for *T. repens* and *T. arvense* respectively (most preferred) in November. At this stage, all species were predominately vegetative, except for *T. dubium* swards which were abundantly flowering with localised areas of senesced herbage (T.M.R. Maxwell, personal field observations, Plate 7.6). The order of relative preference for clover species on Nov 10th was *T. arvense* > *T. repens* > *T. glomeratum* > *T. subterraneum* > mixed species sward > *T. striatum* > *T. dubium*. However, there were no significant differences among species (Table 7.2).
Plate 7.6  Suckling clover *Trifolium dubium* L. in full flower on November 5\(^{th}\) 2009 while all other clover species were at predominately vegetative stages of maturity, Lincoln University, Canterbury, New Zealand.

When all clover swards were reproductive in December, there were significant differences in relative preference among different the clover swards (*P*<0.05, Table 7.2). The order of relative preference was *T. repens* > *T. arvense* > *T. subterraneum* > mixed species sward > *T. striatum* > *T. glomeratum* > *T. dubium*, ranging from 0.065 for *T. dubium* to 0.248 for *T. repens*. *Trifolium dubium* and *T. glomeratum* (0.095) were the least preferred species group, though not significantly different from *T. striatum* (0.141), mixed species sward (0.143), *T. subterraneum* (0.152) and *T. arvense* (0.156). This intermediate group were observed to be close to neutral preference, assuming a neutral relative preference value of 0.142 (1/n; n = 7 species/swards). *Trifolium repens* was the most preferred clover species, being significantly greater in relative preference value than *T. dubium* and *T. glomeratum*, but not significantly greater in preference than *Trifolium striatum*, mixed species sward, *T. subterraneum* and *T. arvense* (Table 7.2).
7.3.4 Relative preference, herbage quality and grazing time

Relationships between relative preference (α), clover chemical components, and grazing time are shown in Table 7.3. Significant effects of chemical components on relative preference and the relationship between relative preference and grazing time are presented in Figures 7.2 and 7.3. Negative correlations were observed between the relative preference value of clover swards and their ADF, NDF, and OM levels (Table 7.3, Figure 7.2a, 7.2b, and 7.2c). Specifically, clover sward relative preference during the December preference test decreased sharply as ADF levels in clover herbage increased from 20 to 29% (Figure 7.2a). Similarly, a sharp linear decrease in relative preference was observed during the December preference test as the level of NDF in clover herbage increased from 23 to 36% (Figure 7.2b). A steady lowering of relative preference value during November and December preference tests was associated with increasing OM levels in clover herbage (Figure 7.2c). The observed negative correlations between relative preference and ADF, NDF, OM, and grazing time were stronger for the December preference test than during the November preference test (Table 7.3, Figure 7.2).

Positive correlations were observed between relative preference for clover swards and their CP, DMD, and ME levels (Table 7.3, Figure 7.2d, 7.2e, and 7.2f). Significant correlations were also evident between relative preference and the time sheep spent grazing a clover sward (Table 7.3, Figure 7.3). Clover sward relative preference increased sharply in association with higher CP (Figure 7.2d) and DMD (Figure 7.2e) levels in clover herbage during the December preference test. Correlation between relative preference and levels of CP and DMD were very weak or non-existent during the November preference test (Figure 7.2d and 7.2e). Increasing relative preference was observed to be associated with increasing levels of ME in clover herbage during the December preference test (Figure 7.2f). In similar fashion, greater relative preference was associated with increased grazing time on clover swards in December (Figure 7.3). Significant correlations between relative preference and CP, DMD, and ME were only present during the December preference test (Table 7.3, Figure 7.2). There was a significant correlation between relative preference and grazing time in December (Table 7.3 and Figure 7.3) with greater relative preference associated with longer grazing time.
Table 7.3 Correlation coefficients between relative preference and sward structure (DMY and SSH) and chemical components (ADF, NDF, WSC, CP, OM, OMD, DMD, ME, and Grazing time) examined in (a) November 10th and (b) December 16th grazing preference tests at field H18, Lincoln University, Canterbury, New Zealand, 2009. NB *** and ** indicate the component being significantly different at \( P \leq 0.001 \) and \( P \leq 0.01 \) respectively, ns = not significant.

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<th>CP</th>
<th>OM</th>
<th>OMD</th>
<th>DMD</th>
<th>ME</th>
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<th>NDF</th>
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<th>CP</th>
<th>OM</th>
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Figure 7.2 Significant correlations between Relative Preference index and chemical (nutritive) components (a) acid digestible fibre (ADF), (b) neutral detergent fibre (NDF), (c) organic matter (OM), (d) crude protein (CP), (e) dry matter digestibility (DMD), and (f) metabolisable energy (ME). Clover herbage harvested on November 10th 2009 are represented by blue symbols, while clover herbage harvested on December 16th 2009 are represented by red symbols.
Figure 7.3 Relationship between Relative Preference index and Grazing Time (minutes). Clover herbage harvested on November 10th are represented by blue symbols, while clover herbage harvested on December 16th are represented by red symbols.
Table 7.4  Sward morphology (leaf, petiole, stem, flower, dead material and non-clover plant matter) of the six clover species prior to grazing preference tests in spring (November 10th) and early summer (December 16th) at field H18, Lincoln University, Canterbury, New Zealand, 2009. NB ***, ** and * indicate the component being significantly different at \( P \leq 0.001, P \leq 0.01 \) and \( P \leq 0.05 \) respectively, ns = not significant. Different letters within the same row denote means that are significantly different @ 5% level.

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<td>12.9 c</td>
<td>15 c</td>
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7.3.5 Clover sward morphology

Sward morphology of each clover species in November and December is shown in Table 7.4. Clover leaf, stem, petiole, flower, dead matter, and other (non-clover) components are expressed as % DM of clover herbage harvested prior to preference test commencement.

Significant differences among the clover species were observed for leaf ($P<0.05$), stem ($P<0.05$), and flower ($P<0.01$) components prior to grazing on Nov $10^{th}$. Leaf DM in clover swards ranged from 34–63%. *Trifolium striatum* swards had the lowest amount of leaf (34%) followed by *T. subterraneum* (38%). The leaf component of *T. dubium* (47%) and *T. repens* (53.5%) accounted for ~ half of their pre-graze herbage DM. *Trifolium arvense* and *T. glomeratum* swards had the largest leaf content at 57% and 63% respectively, though not significantly greater than *T. dubium* and *T. repens* (Table 7.4).

Stem DM ranged from 19–48% on Nov $10^{th}$ with *T. arvense* swards having the lowest amount of stem (19%) followed by *T. repens* (28%). *Trifolium glomeratum*, *T. dubium*, and *T. subterraneum* swards were composed of moderate amounts of stem at 36, 40, and 41% respectively, while nearly half of *T. striatum* herbage was stem at 48% (Table 7.4). *Trifolium subterraneum* and *T. striatum* were the only clover species with petiole (pseudostems) components on Nov $10^{th}$, at 6 and 8% respectively.

Very few flowers were present within clover species herbage on Nov $10^{th}$, with *T. arvense*, *T. repens*, *T. subterraneum* and *T. glomeratum* flowers accounting for 0–1% of total herbage DM. *Trifolium dubium* and *T. striatum* had ~7% flowers. Only three species (*T. subterraneum*, *T. repens*, and *T. striatum*) had dead matter present at 8, 4, and 0.5% respectively on Nov $10^{th}$ (Table 7.4).

Significant differences between clover species for all morphological components (leaf, petiole, stem, flower; all $P<0.001$) and dead matter ($P<0.01$) were observed prior to grazing on December $16^{th}$ (Table 7.4). Less leaf and stem were present within the herbage of all clover species on Dec $16^{th}$. The one exception was *T. arvense* which had a greater amount of stem at 34% compared to 19% on Nov $10^{th}$ (Table 7.4). Leaf components remained dominant for *Trifolium arvense* and *T. repens* at 46 and 37.5% respectively. Flowers became the dominant DM component in *T. striatum* and *T. glomeratum* swards on Dec $16^{th}$ at 51 and 46% respectively, while stem was dominant within *T. subterraneum* and *T. dubium* swards at 29
and 47% respectively (Table 7.4). All species had greater amounts of petiole, flower, and dead matter components (Table 7.4). *Trifolium dubium* had no petiole present on Dec 16th (Table 7.4).

Figure 7.4 presents the relationship between the amount (%) of a morphological component on offer and the level of the component removed for each clover species during the Nov 10th and Dec 16th preference tests. Three clover species showed a strong positive linear relationship between the level of a component on offer and the level of that component removed, i.e. *T. glomeratum*, *T. subterraneum*, and *T. striatum* for % leaf (Figure 7.4a, 7.4b, and 7.4e respectively). This relationship remained strong when the amount of leaf on offer had decreased from Nov 10th to Dec 16th.

Figure 7.4 Relationships between clover plant morphological components (leaf and stem) on offer prior to grazing, and the amount of clover component removed (%) during November and December grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, 2009.
7.3.6 Grazing behaviour

Grazing time of clover was greater on Nov 10\textsuperscript{th} than Dec 16\textsuperscript{th} (Table 7.2). There were significant differences in time spent grazing between clover swards on both dates (Table 7.2). \textit{Trifolium subterraneum} was the clover species sheep spent the most time grazing on Nov 10\textsuperscript{th}, followed by \textit{T. striatum}, mixed species, \textit{T. dubium}, \textit{T. glomeratum}, \textit{T. repens}, and \textit{T. arvense} (Table 7.2). Five weeks later on Dec 16\textsuperscript{th}, \textit{T. repens} was the clover species that sheep spent the most time grazing, followed by \textit{T. subterraneum} and \textit{T. striatum}, then \textit{T. arvense}, mixed species, and \textit{T. glomeratum} with the least amount of time spent grazing \textit{T. dubium} (Table 7.2).

Merino sheep grazing behaviour during the Nov 10\textsuperscript{th} and Dec 16\textsuperscript{th} grazing preference tests is shown in Figure 7.5 where grazing activity (clover species, or grass) and non-grazing activity (idling, ruminating, or walking) is expressed as a percentage of each hour during the preference test. During the first preference test on Nov 10\textsuperscript{th}, the time spent grazing from clover swards decreased steadily during the first hour. In contrast, the time spent grazing grass increased steadily reaching a maximum after two hours before decreasing slightly during the third hour (Figure 7.5). Idling (non-grazing, ruminating, walking) behaviour was limited overall but increased over the duration of the preference test. Rumination increased steadily over the first hour, declined slightly over the second hour and increased to maximum at the end of the preference test (Figure 7.5).

Grazing time on clover decreased more steadily over the first hour in December than during the first hour in November. Further, in contrast to November, sheep grazing time of \textit{T. striatum} and \textit{T. glomeratum} clover swards began to increase again during the second hour with most time spent grazing from \textit{T. striatum} swards. Grazing time of mixed species, \textit{T. arvense}, \textit{T. subterraneum}, and \textit{T. repens} all increased again during the third hour with time spent grazing \textit{T. dubium} remaining low. Time spent ruminating increased to a maximum in the third hour of observation during the December preference test (Figure 7.5).

There was no difference in grazing behaviour between the young (hogget) and older (wether) sheep during both preference tests.
Figure 7.5 Merino sheep behaviour and grazing activity and the time spent grazing from different clover species swards (%) during November and December grazing preference tests at H18, Lincoln University, Canterbury, New Zealand, 2009.
7.4 Discussion

The objective of this experiment was to investigate sheep grazing preference of four adventive annual clover species in comparison to two sown clover species by quantifying change in both sward surface height and sward biomass during grazing events conducted at two different stages of clover plant maturity. Measurement of sward morphology, observation of sheep grazing behaviour, and quantification of time spent grazing different clover species was made to supplement pre-graze and post-graze clover herbage measurements. Using the Chesson-Manly preference index, a relative preference value for each clover species sward at two different periods in time was calculated from pre-graze and post-graze clover biomass measurements. Chemical analysis of clover herbage was then used to explore the hypothesis that relative preference for these adventive annual clover species is different between species, and that relative preference changes with increasing plant maturity. The information gathered here regarding the relative preference value of these four adventive clover species by Merino sheep, as these species change from vegetative to reproductive stages of maturity, is the first of its type in New Zealand.

The results show that there were significant differences in SSH reduction (%), herbage utilisation (%), relative preference, sward morphology, and grazing time between the species investigated. Differences in SSH reduction (%), sward morphology, and grazing time among the clover species were significant when plants were either predominately vegetative or reproductive. Differences among clover species in herbage utilisation (%) and relative preference however, only became significant when these swards reached a predominately reproductive stage of maturity.

7.4.1 Sward surface height dynamics

Reduction in clover sward height was positively related to the pre-graze SSH, reflecting taller swards being more grazed down when most species were at a predominately vegetative stage of maturity on Nov 10th (Figure 7.1a). Sheep prefer to graze taller swards due to easier prehension of the more erect vegetation (Allden & Whittaker 1970), allowing a greater intake rate either because of faster prehension biting rate and/or greater bite mass (Cosgrove & Edwards 2007). *Trifolium dubium* swards were the exception to the strong general trend of taller, erect swards showing greater SSH reduction, such as *T. subterraneum, T. glomeratum,* and *T. striatum.* *Trifolium dubium* swards showed the least SSH reduction during both preference tests despite being amongst the tallest swards, suggesting sheep actively...
discriminated against this species. The weak relationship observed between pre-graze SSH and SSH reduction in Dec 16th reflects the more uniform sward height between the species prior to grazing, and lower preference (greater discrimination) of more adventive clover species by the Merino sheep when these plants were predominately reproductive. Alongside *T. dubium, T. glomeratum* was one of the least preferred clover species in December.

### 7.4.2 Pre-graze biomass, utilisation & relative preference dynamics

The order of largest to smallest clover species sward biomass prior to grazing on Nov 10th primarily reflects the annual and perennial nature of the species investigated, the known agronomic differences between the annual clover species, and the differences in the phenological development characteristics of all the species, specifically the time taken to the start of flowering.

In general, annual plants grow more rapidly than perennial species. Among the annual species investigated *T. arvense* is the latest maturing (flowering) species (K. Pollock, R. Lucas pers. comm.; T.M.R. Maxwell personal field observations), while the perennial *T. repens* has a slower development rate due to greater thermal time requirements for emergence and seedling development in contrast to the adventive species and *T. subterraneum* (Lonati et al. 2009).

*Trifolium dubium* is the fastest maturing species among those investigated, with flowering starting in October, and also with flowering of *T. glomeratum* starting in November (Webb et al. 1988). *Trifolium dubium* reaches peak flowering in November in moist environments (Caradus & Mackay 1989). The timing of flowering follows leaf production in clover species (Boswell et al. 2003). Thermal time requirements of these four adventive species for flowering initiation are unknown. However, *T. dubium* has the lowest thermal time requirement to initiation of stolon development, with the most number of trifoliate leaves on a stolon out of all other annual clover species in this study at >40 leaves/plant compared to 20–30 leaves/plant, under controlled conditions (Lonati et al. 2009). This suggests *T. dubium* would be the first flower out of the clover species investigated here.

Relative preference shown by the sheep for these clover species appears strongly related to nutritive quality characteristics of the clover swards. Increasing concentrations of indigestible components such as lignin and less digestible cellulose (ADF) on Dec 16th decreased the relative preference value of clover herbage suggesting the Merino sheep discriminated more
against species of lower nutritive quality. In contrast, increasing levels of protein (CP), and digestible components in clover herbage, quantified by DMD, ME, and WSC, appeared to increase the relative preference value for clover herbage. This suggests Merino sheep either discriminated significantly less against and/or actively sought out herbage of higher nutritive quality.

This is in accord with the work of Thomas et al. (2010) who found that when herbage quality was low, at reproductive and senesced stages of maturity, Merino sheep selected annual clover plants with characteristics associated with greater nutritive value. Specifically, ADF became a major determinant of preference as plants senesced. Thomas et al. (2010) suggested that with increasing annual clover plant maturity, animals more strongly avoid species with higher indigestible components.

The relationship between relative preference and nutritive characteristics of clover herbage was not strong when clover species were in a predominately vegetative stage of maturity. This suggests that another factor was the primary determinant of sheep grazing preference when clover species were vegetative. Thomas et al. (2010) found that relative preference did not correlate well with nutritive characteristics when herbage quality was high at the vegetative stage of maturity. Furthermore, the Chesson-Manly relative preference index was perhaps not an adequate method to ascertain preference(s) exhibited by the sheep, if any, for these clover species when most species other than *T. dubium* were predominately vegetative in November.

Palatability of clover species herbage may have been the primary determinant/driver of sheep grazing preference on Nov 10th, while chemical composition (nutritive characteristics) of clover species herbage on Dec 16th became the primary driver of preference once most species had become less palatable (lower leaf %, greater flower head %) at the predominately reproductive stage of maturity. Palatability and preference ranking may have major implications in the establishment and persistence of pastures (Colebrook et al. 1990).

Though measurements relating to palatability of forage, such as sheep intake rate of the different clover swards, ratio of prehension to mastication bites, and shear strength of clover leaves and stems were not taken, morphological data was obtained for each clover species. This can offer insight into what clover morphological components were more or less preferred at two different stages of maturity.
7.4.3 Morphology

*Trifolium subterraneum, T. glomeratum, T. striatum* and *T. repens* showed positive linear relationships between sward leaf content and the amount of leaf removed during Merino sheep grazing. When two pasture swards of different characteristics are offered together, sheep prefer to graze herbage that can be consumed at a faster rate (Black & Kenny 1984). The afore mentioned species all showed moderately high to high utilisation and relative preference during the November grazing preference test.

*Trifolium dubium, T. striatum,* and *T. subterraneum* had a higher proportion of stem than the other clover species at the November sampling. However the data presented does not reflect the fact that *T. dubium* was the earliest flowering / maturing species, and was at peak flowering at the time of the November sampling. It is also important to report that the flowers of *T. dubium*, although prolific in number, have a low DM content relative to the stems and relative to the flowers of other species in this experiment (*T. striatum* and *T. glomeratum*). This is a critical factor which must be considered when interpreting this data.

Early flowering in general is a characteristic of *T. striatum, T. glomeratum* and *T. subterraneum* because they are annual species which typically flower early as an adaptive mechanism towards survival in dry environments. All the other species were leaf dominant in November. In contrast, *T. arvense* and *T. repens* are later flowering, reflecting greater thermal time requirements for development, which has previously been demonstrated in literature for *T. repens* (Lonati et al. 2009) and specifically time to flowering for *T. arvense*. There is large ecotypic variation for flowering time for this species in New Zealand (Palmer 1976). Another reason may be that some of these clovers adopt different growth habits in response to immediate soil moisture / climatic conditions, as an alternative survival strategy (See Chapter 2, Table 2.2; Webb et al., 1988).

In December, the proportion of stem and leaf decreased for all species, excluding *T. arvense.* At this point, *T. striatum* and *T. glomeratum* were flower DM dominant, while *T. subterraneum* and *T. dubium* were increasing in flower dry matter. In contrast, *T. repens* and *T. arvense* remained predominately vegetative. This result suggests that as we approach a point of low soil moisture availability, the timing of maturation (species turning predominantly reproductive) is in the order of *T. dubium > T. striatum = T. glomeratum > T. subterraneum > T. repens = T. arvense.*
7.4.4 Grazing behaviour dynamics

In November, sheep in this experiment, when presented with seven different clover sward options, clearly spent more time grazing *Trifolium subterraneum*, followed by *T. striatum*. *Trifolium subterraneum* was also the single dominant species grazed in the first hour of grazing. Following this, clovers of ‘intermediate’ grazing times included ‘mixed species’, *T. dubium* and *T. glomeratum*. This may be explained by *T. subterraneum* and *T. striatum* leaves being predominantly high in the canopy, as indicated by these species having more stem DM (see previous section). *Trifolium subterraneum* also had the highest concentration of WSC of all species, and *T. striatum* had the highest protein content of all species at this time. Further, *T. subterraneum* has the largest leaf size of the annual species, followed closely by *T. striatum*. In combination, these factors likely encouraged higher selection preference for grazing *T. subterraneum* and *T. striatum* at this time. Leaf size and height are factors that determine the acceptability of clover herbage, with ease of prehension being the most important factor controlling consumption of pasture swards (Hill et al. 1995). Hyslop et al. (2003) reported that *T. subterraneum* cv. Leura was the most preferred annual clover species when compared alongside *T. respinatum* and *T. balansae*.

*Trifolium repens* and *T. arvense* were grazed for the shortest period of time. For *Trifolium repens* and *T. arvense*, grazing time was low, likely resulting from lowest DM mass on offer and high relative preference index values. High relative preference was strongly related to vegetative state at the time of grazing, as discussed in the morphology and relative preference section. This in turn suggests that these two species were rapidly grazed to a low residual, at which point the sheep moved on to graze other species. Merino sheep intake rate may have been significantly greater from swards of these two species also.

For the December grazing, there was less variation in the time spent grazing the various species options. Grazing times were in the order of *T. repens* = *T. striatum* = *T. subterraneum* > *T. arvense* > mixed = *T. glomeratum* > *T. dubium*. By December most species were predominantly reproductive showing a spectrum of maturity, with *T. dubium* being most mature and both *T. repens* and *T. arvense* the least (at early flowering stages). All other species were at mid to late flowering stages. This degree of maturity strongly reflected grazing time, which in turn reflected relative preference index. Species such as *T. repens*, *T. striatum* and *T. subterraneum* generally also had higher protein and WSC compared with the other species at this time. These results strongly indicate that the degree of clover maturation is the key factor driving grazing preference of Merino sheep at this time of year. Interestingly,
T. arvense has been shown to contain condensed tannins (Bate-Smith 1973; Sarkar et al. 1976) and at greater amounts than other clover species whose herbage has been found to contain condensed tannins; T. aureum, T. badium, T. campestre, T. micranthum, T. patens, T. spadiceum, and T. dubium (Marshall et al. 1979; Fay & Dale 1993). This may have influenced the grazing behaviour of the sheep and the proportion of biomass intake from this species (Molle et al. 2008). Trifolium dubium has also been shown to contain condensed tannins (Fay & Dale 1993) however this species was least preferred by sheep in this experiment. Comparison of concentration and type of condensed tannins in herbage components (leaf, stem, inflorescences) of these two species at different stages of plant maturity requires further investigation. Condensed tannins in T. arvense and T. dubium may not be qualitatively similar; differences in nutritive value of different clover species herbage may be due to differences in condensed tannin composition (Sarkar et al. 1976). Additionally, Merino sheep are known to be ‘highly selective grazers’ (browsers) in high country environments, whereas other sheep breeds tend to be ‘general grazers’ (E. Gibson and A. Marshall, pers. comm.).

7.4.5 Implications

Colebrook et al. (1990) stated that changes in preference ranking could have considerable influence on clover productivity and persistence under grazed conditions. Specifically, a low preference ranking during early growth would assist a plant to establish within a pasture sward, while a high preference ranking during flowering and set seed (reproductive stages) would considerably reduce persistence in the pasture sward. Results from this experiment would suggest that several clover species with reduced relative preference values at reproductive stages (T. dubium, T. glomeratum, T. arvense) may result in their increased persistence in a grazed pasture sward. The neutral grazing preference of T. striatum throughout the different stages of maturity in this study, may assist its establishment and persistence in a grazed sward. The higher the biomass of an annual plant at the end of the growing season, the greater amount of seed a plant species is likely to produce and thus perpetuate (Broom & Arnold 1986).

Merino sheep grazing selectivity for T. subterraneum or Lolium rigidum gave undesirable plant species such as Capeweed Artotheca calendula and long-beaked storksbill Erodium botrys a competitive advantage in an annual pasture (Broom & Arnold 1986). Selective grazing of some clover species over other clover species may provide a competitive advantage
in the same way, with benefit to a pasture system, as clover species of lower grazing preference at any stage of maturity would be desirable because of N-fixation inputs.

Manipulation of clover diet preference could be used towards increasing long-term sustainability of legume content within the sward (Edwards et al. 2008). Specifically, the breeding or introduction of less preferred legumes in pastures alone, or in combination with breeding for selective and non-selective sheep, is a possible means towards achieving a sustainable and long-term increase in pasture legume content, and the diet of foraging animals. Management (grazing and soil fertility) to promote and sustain the abundance of adventive annual clover species already present in hill and high country pasture systems may be of considerable benefit to pastoral productivity in the long-term through annual N inputs (N-fixation and clover plant decomposition) and availability of palatable herbage during vegetative stages of maturity.

### 7.5 Conclusions

Relative preference of adventive annual clover species changed as plants progressed from predominately vegetative to reproductive stages of maturity. Relative preference index was strongly related to nutritive quality characteristics of digestibility and protein when plants were reproductive.

*Trifolium dubium* was the most rapid maturing and the least preferred species, while *T. repens* was the latest to flower and the most preferred species. *Trifolium arvense* and *T. glomeratum* were the most preferred adventive species in November, but these species became less preferred in December. *Trifolium subterraneum* was moderately high/intermediate in preference value, remaining consistently well utilised during grazing preference tests in November and December. Sheep showed a consistent neutral preference for *Trifolium striatum* and ‘mixed species’ sward during both November and December grazing preference tests.

Adventive annual clover species of lower grazing preference at reproductive stages of maturity may benefit long-term pasture sustainability in summer dry hill and high country pastures via their annual regeneration from seed, DM production, and N inputs.
Chapter 8
General Discussion

This thesis examined:

- Influence of environmental factors (climatic, edaphic, and topographical) on the abundance of adventive annual clover species in South Island hill and high country
- Adventive annual clover species biomass production, botanical contribution, and population dynamics in relation to spring grazing management and superphosphate application at a summer dry high country hill pasture site
- Dry matter production response of adventive annual clover and sown clover species to increasing levels of plant-available soil phosphorus grown in a typical high country soil under glasshouse conditions
- Dry matter production response of adventive annual clover and sown clover species to increasing levels of plant-available soil sulphur and increasing soil pH grown in a typical high country soil under glasshouse conditions
- Grazing preference of Merino sheep for adventive annual clover and sown clover species at different stages of plant maturity growing as pure swards within a perennial grass background
8.1 Summary

Chapter Three – Influence of environmental factors on adventive annual clover species abundance in South Island hill and high country

The objective of this first research chapter was to quantify the abundance of naturalised adventive annual clover species in relation to climate, topography, soil, and farm management on extensive grazing blocks. Site surveys were conducted on two climatically different South Island high country stations; low rainfall (703 mm/year) Mt Grand Station during mid-December, and high rainfall (1665 mm/year) Glenfalloch Station during mid-January. On each station, measurements of grassland species cover, soil depth and fertility, sward height (as a measure of grazing intensity), slope angle, altitude, and aspect were taken within 5 x 5 m quadrats along 200 m long transects at upper, middle, and lower slope positions within three grazing blocks (paddocks ranging from 65–100 ha plus in size). Clover species percentage cover in each quadrat was used as the response variable in fitting a multiple logistic regression model, constructed using altitude, aspect, slope, perennial grass, grazing intensity, Olsen P, sulphate S, and soil pH as predictor variables.

The main findings were:

- Soil moisture, as determined by annual rainfall, appeared to be the main factor determining the presence or absence of naturalised adventive annual clovers in South Island hill and high country.
- Aspect was a dominant factor affecting the abundance of annual legumes with higher abundance of the adventive species *Trifolium striatum* and *T. glomeratum* in sunny, north facing aspects at Mt Grand Station.
- *Trifolium dubium* was the only adventive species observed at Glenfalloch Station.
- *Trifolium striatum* abundance decreased while *T. repens* abundance increased with increasing available soil P.
- In a summer dry environment such as Mt Grand Station, naturalised adventive annual clovers grow in combination where the sown species, white and subterranean clover, remain at low abundance.
Chapter Four – Adventive annual clover species biomass production, botanical contribution, and population dynamics in relation to spring grazing management and superphosphate application at a summer dry high country hill pasture site

The influence of different spring grazing management (continuous, early closure, late closure) and superphosphate (SP) application (low: 75 kg/ha – high: 200 kg/ha) on the resident adventive annual clover species at a mid-altitude (~700 m a.s.l.), north-facing (sunny), moderately steep high country pasture site was investigated over two consecutive growing seasons (October 2008–May 2010). Adventive annual clover species botanical composition and biomass production was measured by visual estimation (%) of pasture species cover and pasture quadrat cuts taken from within 2.5 x 5 m SP fertiliser split plots within 5 x 5 m grazing management treatment main plots during spring, summer, and autumn. Reproductive performance of the resident clover species was measured by seed production counts in summer of the first year, and clover seedling recruitment measured in autumn of both years. The main findings were:

- Variation in seasonal rainfall distribution from year to year, specifically during the spring to early summer period (September–November and early December) was the primary determining factor for adventive annual clover species abundance in north-facing summer dry high country grassland, owing to its effect on soil moisture availability and thus pasture biomass production and associated seed set.
- Spring grazing closure from mid-October positively influenced adventive annual clover species abundance in a moist season. However, this produced no significant increase in seed production and no detectable positive influence on seedling recruitment in the following autumn, or adventive annual clover herbage mass in the following dry spring.
- *Trifolium striatum* was the most dominant clover species, either in moist or dry spring–early summer conditions. *Trifolium glomeratum* and *T. dubium* were secondarily-dominant to *T. striatum* in a moist spring–early summer. The least abundant clover species in either a moist or dry season were *T. arvense*, *T. repens* and *T. subterraneum*.
- Overall, no detectable DM response to superphosphate application was observed by adventive annual clovers suggesting that these species may be adapted to low P and S environments and so require low P and S levels to grow well.
- Seedling recruitment of *Trifolium striatum*, *T. dubium*, and *T. glomeratum* was significantly greater within areas of low SP application.
Chapter Five – Dry matter yield and nutrient uptake response of four adventive annual clover and two sown clover species to increasing levels of plant-available soil phosphorus grown in a typical high country soil under glasshouse conditions

A glasshouse experiment was conducted to quantify the DM response, nutrient uptake and response efficiency of the four adventive annual clover species to increasing levels of available P in a typical low fertility, acidic high country soil (High Country Southern Brown soil). The adventive annual clover species response was compared against that of *Trifolium repens* and *T. subterraneum* as two sown ‘reference’ clover species. The levels of applied P ranged from 30 to 2500 mg P/kg soil; equivalent to 13 to 1105 kg P/ha.

The main findings were:

- *Trifolium subterraneum* ‘Mt Barker’ was the most P responsive clover species showing the largest difference in yield DM response, shoot P concentration, P uptake, and P use efficiency of the species tested.
- *Trifolium arvense* was the most P responsive adventive species with a greater yield DM response and P response efficiency than the perennial *T. repens* ‘Nomad’.
- *Trifolium glomeratum* was intermediate in yield response though more efficient at utilising P at low levels for DM production than *T. repens* ‘Nomad’.
- *Trifolium dubium* and *T. striatum* were the least P responsive legume species.
- The results suggested that two adventive annual species (*T. arvense* and *T. striatum*) have lower P requirements for maximum yield response than *T. repens* and *T. subterraneum*. 
Chapter Six – Sulphur and lime dry matter response of adventive annual clover species grown in a New Zealand high country soil under glasshouse conditions.

The growth response and nutrient uptake of the four adventive annual clover species to increasing levels of applied plant-available S or lime (CaCO$_3$) in a typical low fertility, acidic high country soil (High Country Southern Brown soil) was determined under glasshouse conditions. Again, the adventive annual clover species DM response and nutrient uptake was compared to that of *Trifolium repens* and *T. subterraneum* as ‘reference’ clover species. The levels of applied S ranged from 10 to 240 mg S/kg soil; equivalent to 5 to 132 kg S/ha. Lime was applied at rates of 0, 1, 2, 4 and 8 t lime/ha equivalent.

The main findings were:

- The four adventive annual clover species and *T. subterraneum* were minimally responsive to increasing S levels. The perennial clover *Trifolium repens* ‘Nomad’ was unresponsive to increasing S.

- Naturalised, adventive annual clover species, *T. subterraneum* ‘Mt Barker’ and *T. repens* ‘Nomad’ produced comparably high TDM yields at low levels of Sulphate-S, adding value to the contention that available P level is the key factor (after N) for pasture legume performance in the context of hill and high country soil fertility.

- Under the climatic, soil basal fertility and moisture conditions created in this glasshouse experiment, these species appear to have low S requirements for near optimum production in terms of shoot S concentration and S uptake.

- *Trifolium striatum* and *T. repens* ‘Nomad’ were the only species to show a positive yield response to increasing soil pH levels above the natural acidic level of 5.1. All other species did not show greater TDM yields when soil pH level increased as a result of lime addition.

- *Trifolium arvense, T. subterraneum* ‘Mt Barker’, *T. dubium* and *T. glomeratum* were tolerant of acidic soil pH levels and produced highest TDM yields from pH 5.1–5.5. All adventive annual species and *T. subterraneum* ‘Mt Barker’ produced greater yields than *T. repens* ‘Nomad’.

- Significant negative yield responses were produced by all species at the highest lime rate of 8 t/ha equivalent, likely due to a high soil pH-induced reduction in the availability of P, B and Cu.
Chapter Seven – Merino sheep grazing preference for adventive annual clover and sown clover species at two different stages of plant maturity

The grazing preference of the four adventive clover species and two sown clover species by Merino sheep were investigated in a field experiment whereby pure swards of each clover species were allowed to be grazed at two different stages of maturity. Pre-grazing and post-grazing sward measurements (sward height and biomass) and observations of grazing behaviour, specifically time spent grazing different clover species swards on offer, were made when most species were at predominately vegetative and reproductive stages of plant maturity. A relative preference index for each species was calculated from herbage mass on offer and herbage offtake by sheep, relative to the offtake of all other species on offer. Herbage chemical composition analysis of each clover species was conducted via NIRS to determine nutritive quality at the two different stages of maturity.

The main findings were:

- Relative preference of adventive annual clover species changed as plants progressed from predominately vegetative to reproductive stages of maturity.
- Relative preference index was strongly related to nutritive quality characteristics of digestibility and protein when plants were reproductive.
- *Trifolium dubium* was the most rapid maturing and the least preferred species, while *T. repens* was the latest to flower and the most preferred species.
- *Trifolium arvense* and *T. glomeratum* were the most preferred adventive species in November, but these species were less preferred in December.
- *Trifolium subterraneum* was intermediate in preference value.
- Merino sheep showed neutral preference for *Trifolium striatum* and ‘mixed species’.
8.2 General Discussion

The economic viability of high country pastoralism rests on productive pastures in the remaining middle and lower altitude zones, specifically for lactating ewes during spring and their weaned lambs in summer. Introducing, increasing, and enhancing legume content within pastures has been the main progression of primary pasture improvement in NZ through the decades since the advent of aerial topdressing and oversowing. The main contention of this thesis was that white clover *Trifolium repens*, as the most commonly sown clover species within NZ, has poor persistence in summer dry hill and high country areas and especially on northerly-facing aspects (Knowles et al. 2003; Power et al. 2006; Maxwell et al. 2010). In contrast, annual species of naturalised adventive grassland legume flora in the hill and high country of NZ’s South Island (predominately *Trifolium arvense*, *T. dubium*, *T. glomeratum*, and *T. striatum*) are commonly present to locally abundant (Boswell et al. 2003; Power et al. 2006; Maxwell et al. 2010).

Information about the abundance of these naturalised adventive (volunteer) annual clover species within climatically different South Island hill and high country areas; their DM productivity, botanical composition, and population dynamics in a typical summer dry hill side pasture, their DM response to P, S, and lime while growing in a typical high country soil (low fertility and acidic), and their grazing preference by the predominant sheep breed in the high country, the Merino, was unknown or limited and lacking in the literature (Boswell et al. 2003).

The primary purpose of this thesis therefore was to investigate the ecology of the four most prevalent species of naturalised grassland legume flora found in the South Island hill and high country of NZ. With this purpose in mind, the primary aim was to attempt to address livestock feed production issues associated with decreasing high country pastoral land area and associated land areas (lamb and cattle finishing) as a result of high country land tenure review (O’Connor 2003) and dairy industry expansion (FAO 2009; Maxwell et al. 2010), through the provision of practical implications and/or management advice derived from the main research findings/conclusions of this thesis.

The high country station site surveys (Chapter 3) provided new information on the locality, abundance, and ecophysiology of these adventive clover species in South Island hill and high country areas. These site surveys highlighted that soil moisture (by implication annual rainfall and hill slope aspect) and soil fertility are primary drivers of adventive annual clover presence
and abundance. It was stipulated from these findings that adventive pasture legumes exhibit regeneration and persistence strategies (avoidance of grazing, high seed production, tolerance of low soil pH, S and P) that allow them to regenerate and grow on dry hill and high country pastures. Such strategies of persistence and regeneration could be exploited in breeding programmes for dry hill and high country agroecosystems. Furthermore, these species may contribute significantly to dry hill and high country through pasture production and nitrogen inputs. However, there was limited data on these aspects. Chapter 4 provided data on adventive annual clover productivity.

The two year grazing management and superphosphate field experiment at Mt Grand Station provided new information on the DM productivity of adventive annual clover species in a typical summer dry pasture environment within the mid altitude zone. This experiment also provided information about the order of prevalence of the four species at this site in terms of pasture botanical composition. Spring grazing exclusion and superphosphate fertilizer application had no to little short-term impact/influence on the abundance of these adventive annual pasture legumes, persistent in grazed summer dry NZ South Island hill and high country. Results from this chapter suggest that reproductive success (viable seed production and seedling recruitment) of the most prevalent adventive species at this site were unaffected by the continuous grazing pressure that occurred during the study period. The resident adventive clover species at this site exhibited regeneration and persistence strategies allowing them to annually regenerate, grow, and produce seeds within the annually-variable period of adequate soil moisture/fluctuating growing season duration of this summer dry climatic zone. These species appear ecological successful in dry, extensively grazed hill and high country pastures, and their presence appears strongly attributable to their lifecycle fitting the microclimate variations (Olivier & Medail 1997).

Considering the large difference in adventive clover biomass production between the first and second spring-early summer periods, and fluctuations in herbage biomass produced from the suite of adventive legume flora at Mt Grand Station preceding and subsequent to this study (K. Pollock, R. Lucas, J. Moir pers. comm.), hindsight suggests a two year sampling period was not a long enough time period to fully explain the nature and dynamics of the annual regeneration pattern and magnitude of adventive annual clover sward contribution/component in such a climatically-variable location. The results obtained over the spring, summer, autumn, winter of 2008–2009 and over the spring, summer, and autumn of 2009–2010 are a relatively short measurement period. Additionally, a two year sampling period may not have
been long enough to allow the full adventive annual clover herbage response to superphosphate application under field conditions to be observed.

Glasshouse investigation into adventive annual clover P requirements (Chapter 5) provided new information on how the four adventive species responded to increasing P availability in a typically low fertility high country soil, determining the level of P required by each species to produce optimum DM under optimum soil moisture, temperature, and soil macro and micro nutrient conditions generated in the glasshouse. This chapter also provided additional new information on *T. subterraneum* ‘Mt Barker’ DM production response to increasing P availability in a NZ high country soil. Of the four adventive species studied, *Trifolium arvense* shows potential for further P response investigation under field conditions, as well as germplasm improvement, and subsequent introduction into low fertility, summer dry hill and high country grassland to promote the spread of this N-fixing species already existing in such agroecosystems.

Clover germplasm improvement research in New Zealand, so far, has not included adventive annual clover species in plant breeding programmes. Following the path of Australian pasture legume improvement programmes in recent times (Nichols et al. 2007), it may be strongly argued that a necessity exists for an adventive annual clover species breeding programme in New Zealand. Based on the results of this doctoral study of persistence and regeneration strategies in summer dry environments, tolerance of low fertility (P and S), acidic soil conditions, and reduced grazing preference at reproductive stages of plant maturity shown by these species, the breeding programme purpose would be to improve the agronomic capability of these hardy, naturalised clover species towards greater dry matter production, herbage quality (at vegetative stages), and N inputs for summer dry hill and high country environments. This should perhaps be carefully considered by plant breeding groups in light of the low abundance of sown legumes in hill and lower altitude high country (Maxwell et al. 2010; T.M.R. Maxwell; Chapter 4), the limited performance of the current summer dry variety of *Trifolium repens* cv. Nomad (T.M.R. Maxwell, unpublished data), reduced persistence of *T. subterraneum* cv. Mt Barker (Power et al. 2006), and slow progress with development of perennial species hybrids (*T. repens* x *T. ambiguum*) for summer dry zones (Widdup et al. 2003).

Current research is being conducted on alternative/novel annual clover species, and perennial forage options of Lucerne *Medicago sativa* and Lupins *Lupinus* sp. establishment in summer
dry South Island hill and high country areas within the Tekapo, Omarama, and Benmore districts of the Mackenzie Basin region. Oversowing annual clover species, alone or in combination with superphosphate topdressing, or pre-sowing herbicide spraying of resident vegetation, has occurred at both large scale (whole hill block) and small scale (0.35ha experimental plot) levels. Species being investigated for introduction into this climatically-variable hill and high country zone are gland clover *T. glanduliferum*, balansa clover *T. michelianum*, and varying varieties of early to late flowering *T. subterraneum* from Australian seed lines; Trikkala, Seaton Park, and Rosabrook (R. Lucas, D.J. Moot, A. Black, K. Pollock, J. Moir, T. Ryan-Salter, pers. comm.). Preliminary results are encouraging. However, the seedling abundance of these species has been small relative to that of resident adventive annual clover species at Omarama Station in late September (early spring) where *T. arvense* and *T. striatum* were common to locally abundant on 450–500 m a.s.l. hill slopes (T.M.R. Maxwell personal observations) over which *T. michelianum* and *T. glanduliferum* had been aerially broadcast-oversown the previous late summer/early autumn, following a high spring and early summer rainfall year; 2011. Similar observations were made at Bog Roy Station where *T. michelianum*, *T. glanduliferum* and three *T. subterraneum* varieties had been oversown onto summer dry hill country (T.M.R. Maxwell, personal observations).

Glasshouse investigations into S and lime requirements (Chapter 6) highlighted that these four adventive annual species, along with *T. subterraneum* ‘Mt Barker’ and *T. repens* ‘Nomad’ required low levels of available S to produce maximum DM, reflecting how strongly clover production is driven by the availability of P in soil. The results of this chapter, namely good herbage yield production under low Sulphate-S and low pH levels, warrant further investigation into the S and lime responses of these species under field conditions, to ascertain if the potentially beneficial agronomic attributes of both *T. subterraneum* ‘Mt Barker’ and the adventive annual legumes can be exploited for livestock pasture feeding by increasing the spread and presence of these species in summer dry, hill and high country environments.

Boswell et al. (2007) reported a $5^{1/2}$ fold increase (10.5 increasing up to 55.3 kg N/ha) in N fixation by *Trifolium arvense* in response to S addition (25 kg/ha of S fertiliser) during a high spring–summer rainfall year in the Mackenzie Basin region. Though N fixation by *T. arvense* and the three other adventive annual clover species was not measured in this doctoral study, this is a clear avenue for further research given the value of using S fertiliser application as a means to improving soil N levels where adventive annual clover species are abundant in hill and high country slopes (Boswell et al. 2007). Additionally, there is scope to increase pasture
production and N inputs in summer dry hill and high country areas through a combination of S topdressing together with adventive annual clover species seed oversowing.

Chapter 7 showed that Merino and Merino Dorset cross sheep exhibited reduced grazing preference for these adventive annual species once these species reached a predominately reproductive stage of maturity. The implications of this for hill and high country grazed pastures are positive in terms of sustainability of clover content (Edwards et al. 2008), whereby reduced grazing pressure for these adventive species at reproductive stages due to being less preferred by sheep may benefit their production of viable seed, and increase cycling of N through annual decomposition of annual clover herbage that has escaped grazing (Goodman 1988; Ledgard & Steele 1992).

The strategic use of coarse salt (NaCl) application is a tool for pasture management in hill and high country. Salt fertiliser application has been shown to enhance grazing pressure by Merino sheep on sodium (Na)-deficient inland high country pastures, resulting in soil disturbance, reduced resident grass competition, and the creation of up to 50% bare ground patches (Gillespie et al. 2006). Such areas of bare ground are ideal colonisation sites for seeds of adventive annual clover species; salt application increased oversown seed establishment of plantain Plantago lanceolata and Trifolium michelianum (Gillespie et al. 2006). As salt application can be used to manipulate grazing management, a combination of mob stocking with sheep and salt application is a means to utilise pasture areas of less preferred reproductive adventive clover biomass accumulation via high, salt-driven, grazing pressure with desired consequences to the surrounding pasture plant community of increased nutrient recycling and increased dispersal of clover seed via animal dung deposition (Boswell et a. 2007).

There has been suggestion that some of these adventive annual clover species have been spreading in the South Island hill and high country areas within the last three decades. Specifically, Trifolium arvense was absent on a north-facing slope on Tara Hills High Country Research Station in 1966 (Douglas and Kinder 1975) but is now a resident species at this 850 m altitude site (Boswell et al. 2007). Whether the other adventive clover species are also spreading in the hill and high country, or have occupied all favourable niches already is unknown and is an avenue for further research. Determining the habitat conditions in which plant community structure is regulated more by local ecological interactions or by the pool of available colonists is an important goal in grassland ecology (Foster 2001), with relevance to
NZ hill and high country management, for both pasture production and biodiversity conservation purposes.

Herbaceous legumes have been reported to be better invaders (colonisers) as seeds than perennial grass species in prairie grassland within native oak savanna in Minnesota, USA (Tilman 1997). The total plant community cover increased with the number of species added, however pre-existing (resident) species cover was found to be independent of the number of species added as seed; the new species filled previously empty sites (Tilman 1997).

Possible modes of dispersal for clover species include endozoochory (seed dispersal through dung deposition) (Russi et al. 1992; Traba et al. 2003) and anemochory (wind/aerial dispersal; all four adventive species are aerial seeders with their numerous seeds less than <2 mm in diameter (Table 2.1), (Boswell et al. 2003). Russi et al. (1992) found that seed recovery following sheep ingestion from Mediterranean grassland in north-west Syria was greater for clover species of smaller seed sizes (such as *T. campestre*); the level of seed recovery was inversely proportional to seed size. Endozoochory has been reported to increase species richness of abandoned grassland areas adjacent to cattle-grazed grassland, and is an effective mechanism of viable seed dispersal in Mediterranean grasslands (Traba et al. 2003). *Trifolium striatum* and *T. glomeratum* were clover species that showed a clear increase in abundance following dung addition.

### 8.3 Future Research

- Influence of increasing levels of P, S, and lime on adventive annual clover species DM production under hill and high country field conditions
- Examination of N fixation capacity of *Trifolium striatum*, *T. glomeratum*, and *T. dubium* in summer dry hill and high country pasture in isolation, and in relation to P and S fertility
- Quantifying N inputs into a typical hill and high country pasture community during and/or following a moist spring season
- Investigation into Merino sheep performance/live weight gain on adventive annual clover species at a vegetative stage of maturity in early–mid spring
- Germplasm improvement of adventive annual clover species via a plant breeding programme for summer dry hill and high country pastures
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Publications during the course of the study
