A comparison of Caucasian and white clovers in temperate pastures

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A series of three main experiments aimed to define the place of Caucasian clover (*Trifolium ambiguum* M. Bieb) with respect to white clover (*T. repens* L.) in temperate New Zealand pastures. Experiment 1 used sheep liveweight gain (LWG) on Caucasian clover–ryegrass (*Lolium perenne* L.) (CC–RG) and white clover–ryegrass (WC–RG) pastures to assess the relative clover performance under high (High-F: Olsen P 20 μg/ml, sulphate-S 12 μg/g) and low (Low-F: Olsen P 11 μg/ml, sulphate-S 7 μg/g) soil fertility conditions. Mean annual sheep LWG on CC–RG was 1178 kg/ha at High-F and 1069 kg/ha at Low-F, and both treatments exceeded WC–RG by ~9%. LWG on CC–RG averaged 141 g/head/d compared with 129 g/head/d on WC–RG. The greater sheep LWG per hectare was attributed to the higher mean clover content (20%) for CC–RG than WC–RG (10%) pastures of similar nutritive value.

Dry matter (DM) production and nitrogen (N) yield (DM production × % N) from CC–RG and WC–RG pastures was used to assess the relative seasonal clover performance under High-F and Low-F conditions. In High-F, total N accumulation rates (grass plus clover) for CC–RG were 0.5–0.7 kg N/ha/d higher than WC–RG from October to February, due to double the rate of N accumulation by Caucasian clover. Similarly in Low-F clover N accumulation rates were 50–120% greater in CC–RG than WC–RG. In High-F spring clover production rates increased by 3.2 kg DM/ha/°C for Caucasian clover compared with 1.3 kg DM/ha/°C for white clover as 100 mm soil temperature increased from 6 to 15 °C. In autumn, DM production of Caucasian clover decreased more than white clover as soil temperatures dropped from 16 to 8 °C. In High-F, annual total and clover DM yields from CC–RG were 17.5 and 4.4 t/ha, respectively, compared with 16.2 and 2.1 t/ha from WC–RG. Both pastures produced ~15.6 t/ha of total DM at Low-F, but clover DM was greater for CC–RG at 3.9 t/ha

than WC–RG at 2.2 t/ha. Thus, Caucasian clover production was greater than white clover during spring and summer with the greatest advantage under High-F conditions.

In Experiment 2 the DM production and water use efficiency (WUE) of each species were compared under full irrigation and dryland (non-irrigated) conditions. In their third year, sown monocultures of Caucasian clover produced 11.9 t DM/ha when irrigated and 9.3 t DM/ha under dryland conditions. Both of these treatments exceeded white clover by ~2.5 t DM/ha due to ~23 kg DM/ha/d higher production rates for Caucasian clover during spring and summer. Specifically, production rates of irrigated treatments increased by 11 kg DM/ha/°C for Caucasian clover compared with 8 kg DM/ha/°C for white clover as mean daily air temperature increased from 8 to 16 °C. In late summer/autumn production rates of Caucasian clover decreased more than white clover when air temperature dropped from 16 to 9 °C. Both species had similar water use under irrigated (~913 mm) and dryland (~740 mm) conditions. This gave mean WUE values of ~13 and 9 kg DM/mm of water for dryland Caucasian and white clovers, respectively.

Growth (leaf photosynthesis rate) and development (leaf appearance rate on a shoot apex) responses of each species to temperature and water status were also measured in Experiment 2. Leaf photosynthesis rates were ~6 μmol $CO_2/m^2/s$ higher for Caucasian than for white clover irrespective of measured air temperatures (7–28 °C) and soil water from 1.00–0.39 of water holding capacity (WHC; 580 mm to 1.7 m depth). Both clovers had similar ranges of optimum temperature (21–25 °C) and soil water (1.00–0.86 of WHC) for leaf photosynthesis. Equally the phyllochron was similar between the two species (126 °Cd), but the higher base temperature (T_b) for Caucasian (5 °C) than white (1 °C) clover would mean Caucasian clover is slower to recover to canopy closure post-grazing. Experiment 2 highlighted the potential of Caucasian clover to increase spring and summer clover production, in combination or as the sole legume species in both irrigated and dryland grass/clover pastures.

Experiment 3 evaluated the impact of spring and autumn sowing and ryegrass seeding rate on the establishment of Caucasian and white clovers. In spring of the second year, white clover content was >15% when sown with 3–12 kg/ha of ryegrass on 24 September (SD1), 9 November (SD2), or 4 February (SD3), but less than 9% when sown on 31 March (SD4). Caucasian clover never exceeded 9% in any treatment. Sowing on SD1–3 with 3–12 kg/ha of

ryegrass gave the most successful establishment of white clover but only Caucasian clover sown alone in spring produced an adequate legume content in the following spring.

Growth and development characteristics responsible for slow establishment of Caucasian clover were identified in a controlled environment study. A T_b of <4 °C was found for all three species. The thermal time (Tt) requirement for 75% germination was lower for Caucasian (46 °Cd) and white (40 °Cd) clovers than ryegrass (76 °Cd). All three species required ~112 °Cd for 50% emergence and ~214 °Cd for first leaf appearance. The phyllochron for primary stem leaves was slower for Caucasian (109 °Cd) than white (94 °Cd) clover and ryegrass (101 °Cd). Axillary leaves and tillers of ryegrass first appeared after 373 °Cd compared with 440 °Cd for axillary leaves and 532 °Cd for stolons of white clover. In contrast, axillary leaves of Caucasian clover first appeared after 990 °Cd and crown shoots first appeared after 1180 °Cd. Consequently, white clover and ryegrass plants had more leaves (~15.2 /plant) and faster shoot relative growth rates (~0.062 mg/mg/d) than Caucasian clover (5.1/plant and 0.049 mg/mg/d, respectively). Small differences in root/shoot ratio between species were considered to be a minor contributor to slow establishment of Caucasian clover. Slow establishment of Caucasian clover was explained by its delayed axillary leaf and shoot development, and resultant slow relative growth rate compared with white clover and ryegrass. Successful Caucasian clover establishment in fertile soils is therefore most likely to occur in the absence of competition from either ryegrass or white clover.

Key words: clover content, dryland, establishment, irrigation, nitrogen fixation, nitrogen yield, perennial ryegrass, photosynthesis, phyllochron, secondary shoot development, sheep liveweight gain, soil fertility, *Trifolium ambiguum* M. Bieb, *Trifolium repens*, water use efficiency.

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Chapter 1

General introduction

1.1 Background

Legumes in pastures reduce reliance on fertiliser nitrogen (N) inputs, complement seasonal growth patterns of grasses, and enhance animal performance. However, a lack of clover persistence in pastures is recognised as a major limitation worldwide (Marten *et al.*, 1989). White clover is the legume that is used most commonly across all livestock production systems and regions in New Zealand (Caradus *et al.*, 1995). It is suited to moderate to high fertility soils and moist conditions. However, white clover does not persist well on low fertility soils and in drought conditions (Woodfield and Caradus, 1996). As a consequence, many pastures in New Zealand are legume deficient and N inputs, pasture production and animal performance are all below the site potential (Caradus *et al.*, 1996; Ettema and Ledgard, 1992).

Caucasian clover is a rhizomatous perennial legume with a potentially wide range of adaptation in temperate pastures (Bryant, 1974; Taylor and Smith, 1998). Exceptional winter hardiness, persistence in low-input conditions (Daly and Mason, 1987; Virgona and Dear, 1996), tolerance to drought (Watson *et al.*, 1998; Woodman *et al.*, 1992), and tolerance to frequent defoliation (Allan and Keoghan, 1994; Peterson *et al.*, 1994a) have increased interest in the potential of Caucasian clover as a component in pasture-based livestock production systems. These characteristics have been attributed to its extensive root and rhizome system (Forde *et al.*, 1989; Peterson *et al.*, 1994b).

Caucasian clover shares many attributes with white clover, including its high nutritive value (Allinson *et al.*, 1985). Its major distinction from white clover is that it perenniates underground vegetatively with rhizomes rather than surface stolons (Forde *et al.*, 1989). It also forms an extensive taproot system that has been shown to persist for over 13 years (Strachan *et al.*, 1994) whereas the taproot of white clover dies within 2 years (Brock *et al.*, 2000; Westbrooks and Tesar, 1955). These morphological characteristics confer an advantage to Caucasian clover over white clover in the tolerance of drought and defoliation. However, Caucasian clover has a disadvantage in that as a seedling a greater proportion of carbon is partitioned below ground making it less competitive for light and therefore slower to establish than white clover (Forde *et al.*, 1989).

An important research goal is to define the range of adaptation of Caucasian clover so its potential to replace or complement reliance on white clover can be maximised. The main role of Caucasian clover may be as the long-term legume component of permanent pastures where abiotic (e.g. temperature, water status, soil fertility) and/or biotic (e.g. grazing management, pests, diseases) factors limit white clover productivity and persistence. High country experiments have confirmed the superiority of Caucasian clover over white clover for persistence in infertile soil (Daly and Mason, 1987) and dryland conditions (Woodman *et al.*, 1992) and in a range of grazing managements (Allan and Keoghan, 1994). These results prompted further studies that have shown Caucasian clover out-performed white clover in irrigated (Moss *et al.*, 1996) and dryland (Watson *et al.*, 1996) conditions in more productive lowland ryegrass-based pastures. However, in each example the influence of environmental factors was expressed either in isolation or in terms of the effect on growth or abundance of Caucasian clover. This approach limits the application of results to zones outside the experimental area. Examining the physiological basis for responses provides greater insight and explanations and offers confidence for recommendations of the suitability and role of Caucasian clover for New Zealand pastures in general.

Any advantage in Caucasian clover would be shown by a higher legume content compared with white clover grown in the same environmental conditions. Assuming a similar nutritive value, any increase in legume content should also increase animal performance (Askin *et al.*, 1987; Hyslop *et al.*, 2000; Stevens *et al.*, 1993). Indeed this will depend on the seasonal growth pattern and ability to fix N_2 in response to abiotic/biotic factors. Caucasian clover is winter dormant but displays greater warm season growth and potentially has higher water-use efficiency than white clover. By comparing the major growth (photosynthesis) and development (leaf appearance) characteristics of Caucasian and white clovers, the species-specific responses to temperature and soil moisture can be quantified physiologically to aid the interpretation of their range of adaptation.

Despite its positive attributes in established pastures, the slow establishment of Caucasian clover is a major problem limiting the widespread use of this legume. The initial slow production rate has been attributed to the development of an extensive root and rhizome system (Forde *et al.*, 1989; Hill and Mulcahy, 1995; Taylor and Smith, 1998). This reduces the competitiveness of Caucasian clover seedlings with associated species for light during the establishment phase of a pasture. The rate of seedling canopy development may also be a major factor determining the successful establishment of this species. The physiological parameters that affect the rate of seedling canopy growth and development can be quantified and used to assist decisions on time of sowing and compatibility of other species to be included in pasture seed mixtures with Caucasian clover. Identifying the physiological basis for the lack of competitiveness at establishment should allow strategies to be developed to increase establishment success of Caucasian clover and consequently increase its contribution to pasture productivity in subsequent years.

1.2 Research objectives

The general aim of the research reported in this thesis is to define the range of adaptation of Caucasian clover compared with white clover in temperate pastures. To achieve this, the similarities and contrasts between the two species, in relation to the main abiotic constraints of temperature, water status, soil fertility, and grazing management are quantified. Emphasis is also placed on measuring the competitive ability of the clover seedlings to allow balanced legume/grass pastures to be established. Underlying these aims is the assumption that the main role of Caucasian clover would be the persistent perennial legume component of permanent pasture in environments where white clover suffers from abiotic and/or biotic stress. Furthermore, where stress on white clover is moderate and periodic, Caucasian clover may be complementary to white clover. Based on these assumptions the following objectives were developed:

1. To quantify annual and seasonal production of Caucasian and white clovers under high and low soil fertility (P and S) conditions in an intensively grazed perennial pasture. Specifically, to measure sheep performance and clover contribution when fertility and temperature (seasonal) varied but moisture was non-limiting. The assumption that N would be non-limiting, due to N_2 fixation, was also tested.
2. To calculate the temporal patterns of water use and extraction of Caucasian and white clovers in irrigated and dryland conditions when fertility was non-limiting. This allows the adaptability of both clovers to dryland conditions to be assessed.

3. To define the optimum temperature range for photosynthesis of Caucasian and white clovers grown in non-limiting moisture and soil fertility (P and S) conditions. This will allow seasonal patterns of production in Objectives 1 and 2 to be related to physiological responses.
4. To devise sowing strategies which allow Caucasian and white clovers to become established in permanent pastures. This requires an understanding of the physiological basis for the competitive ability of each species at establishment.

1.3 Thesis structure

This thesis is presented in eight chapters (Figure 1.1). In Chapter 2, literature concerning the place of white and Caucasian clovers in New Zealand pastures is reviewed. Chapter 3 reports on the first 3 years of measured animal performance in a grazing experiment that assessed seasonal Caucasian clover content relative to white clover (Objective 1, Section 1.2). This is supported by an investigation of the N yield and dry matter productivity of Caucasian and white clovers to identify any seasonal differences in growth patterns in Chapter 4 (Objective 1, Section 1.2). In Chapter 5, a water use experiment is described in which the seasonal water use and extraction of Caucasian and white clovers in irrigated and dryland conditions were compared (Objective 2, Section 1.2). Physiological explanations for differences in observed patterns of seasonal production between species are also presented in Chapter 5 in response to temperature and soil moisture (Objective 3, Section 1.2). Chapter 6 describes an experiment in which the establishment of Caucasian and white clovers was compared at different sowing dates with different sowing rates of ryegrass (Objective 4, Section 1.2). This led to a series of complementary controlled environment experiments to investigate the influence of grass competition on seedling development and to provide a physiological explanation for differences in establishment success observed between the two clover species (Chapter 7). Finally, in Chapter 8 the results are drawn together and compared with those previously reported in the literature to provide general guidelines for the successful inclusion of Caucasian clover in New Zealand pastures.

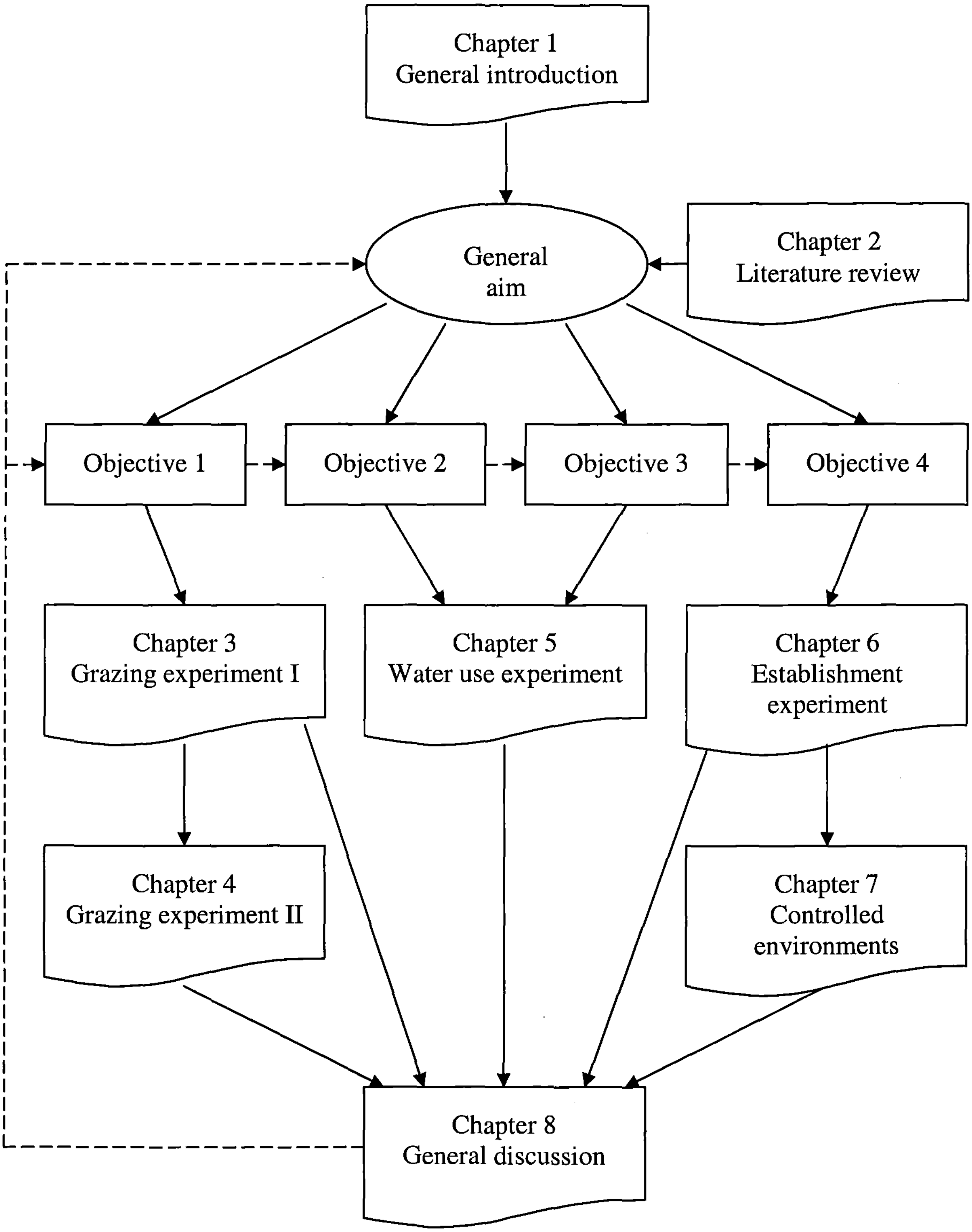

Figure 1.1 Diagrammatic representation of the relationship of each chapter to the general aim and main objectives of the research presented in this thesis.

Chapter 2

Literature review

2.1 Introduction

This chapter compares the responses of white and Caucasian clovers in temperate environments. It acknowledges the international literature reviewed by Frame and Newbould (1986) on white clover and by Taylor and Smith (1998) on Caucasian clover. However, it places more emphasis on New Zealand conditions within the context of the study objectives outlined in Chapter 1.

Initially, the review covers the growth and development characteristics of the two species, and the environmental (temperature, moisture, and soil fertility) and management (grazing or defoliation) factors which determine the suitability of white and Caucasian clovers for a particular environment. Emphasis is given to the agronomic responses and utilisation of each species. The establishment ability of each species is also reviewed, and the development of management strategies for successful establishment of clover species in permanent pastures is outlined. Finally, the review focuses on how an understanding of the plant growth and development responses to temperature and water status would improve the definition of Caucasian clover's range of adaptabion.

Wherever possible, literature is based on comparison of the two species. However, on occasion the lack of information on Caucasian clover is supplemented by data from other temperate pasture species. Furthermore, the species *T. ambiguum* has diploid (2n = 16), tetraploid (2n = 32), and hexaploid (2n = 48) forms, with the hexaploid generally most productive (Taylor and Smith, 1998). Therefore, this review also concentrates on comparisons of hexaploid cultivars of Caucasian clover (e.g. 'Endura', 'Monaro', and 'Rhizo') with cultivars of white clover commonly used in New Zealand (e.g. 'Grasslands Huia', 'Grasslands Demand', and 'Grasslands Kopu').

2.2 Plant growth and development characteristics

The growth and development characteristics of forage legumes in relation to competition and persistence have been reviewed previously (e.g. Forde *et al.*, 1989; Sheath and Hay, 1989). Thomas (2003) divided legume growth and development into four phases: early seedling development, development of the mature plant form, flowering, and post-flowering. The aim of this section is to provide an understanding of the relationship between vegetative growth and development (i.e. the first two phases) of white and Caucasian clovers and their establishment and productivity in temperate environments.

2.2.1 Early seedling development

The pattern of seedling development immediately after germination is very similar in white and Caucasian clovers (Figure 2.1). Germination leads to the emergence of a seminal (seedling) root and two cotyledons. A terminal shoot bud is located between the cotyledons and two axillary cotyledonary buds sit either side of this at the base of the cotyledons. The terminal bud rapidly grows to produce leaves attached to a primary stem. Each leaf subtends an axillary bud. A second phase of development is marked by an increase in the rate of leaf appearance as leaf primordia on the axillary buds appear (Thomas, 1987b; Thomas, 2003).

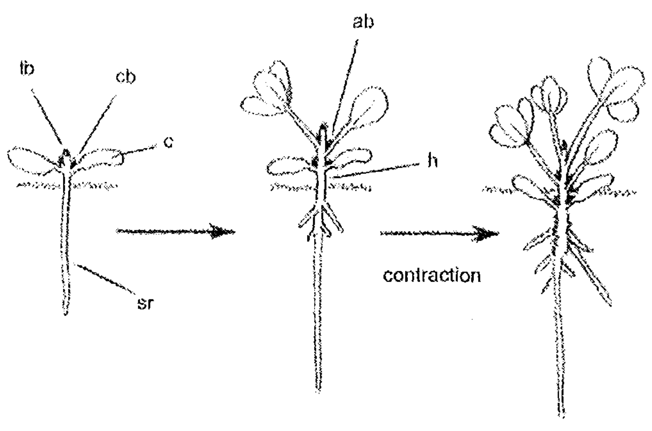

Figure 2.1 Diagram showing early development of a white or Caucasian clover seedling. tb = terminal bud, ab = axillary bud, c = cotyledon, cb = cotyledonary bud, h = hypocotyl, sr = seminal root (Thomas, 2003).

Once the seminal root is established, its upper region, and the hypocotylar region of its stem, contracts, pulling the cotyledonary buds and lowermost axillary buds below the soil surface (Thomas, 2003). The degree of contraction has been reported to be ~5 mm in white clover

(Mitchell and Nelson, 2003), and observed but not quantified in Caucasian clover (Genrich *et al.*, 1998).

2.2.2 Development of the mature plant form

2.2.2.1 Branches

Continued development of the seedling in both species leads to the formation of an initial crown of branches growing on top of a central taproot. The first of these branches grow from the cotyledonary buds and lowermost axillary buds on the primary stem (Figure 2.1). They have alternately arranged leaves each of which subtends an axillary bud. Most axillary buds develop early into branches, but some remain inactive with the potential to develop into branches later. The timing of branch appearance has been related to apical dominance in white clover (Thomas, 1987b). The primary stem elongates very little (10–20 mm) and stops growing after producing about 10 leaves (Figure 2.2), giving the seedlings an initial rosette habit (Thomas, 1987b; Thomas, 2003).

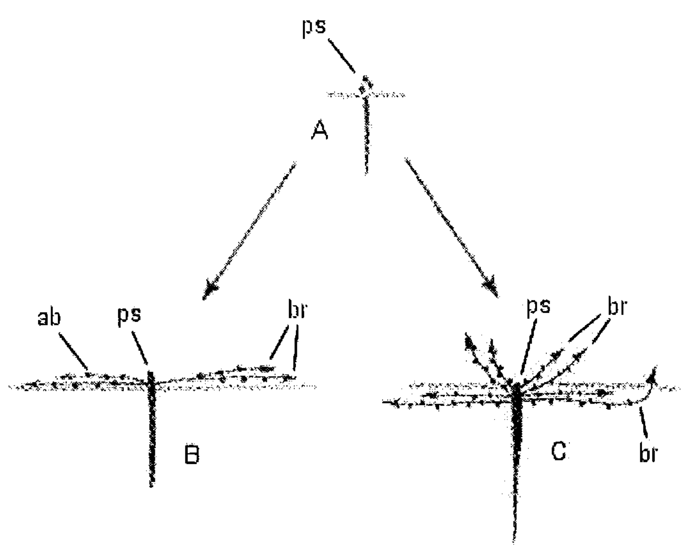

Figure 2.2 Diagrammatic representation of the development of the mature plant forms in white and Caucasian clovers. ps = primary shoot, ab = axillary buds, br = branches. A = generalised seedling, B = white clover, C = Caucasian clover (adapted from Thomas (2003)).

Development beyond this initial rosette phase differs greatly between the two species (Figure 2.2). In white clover, non-flowering branches elongate horizontally above ground to form lateral stolons (Figure 2.3). The stolon is the basic structural unit of the mature white clover plant. It consists of a series of nodes separated by internodes which form as a result of growth from the terminal bud. Each node bears a photosynthetic leaf with an erect petiole, two root primordia, and an axillary bud. The axillary bud is capable of forming either into a lateral stolon, or into an inflorescence (Thomas, 1987a). Stolon growth is dependent on carbon from the photosynthetic leaves closest to the terminal bud, while older leaves supply carbon predominantly to the nodal root systems at their nodes of origin (Thomas, 1987b).

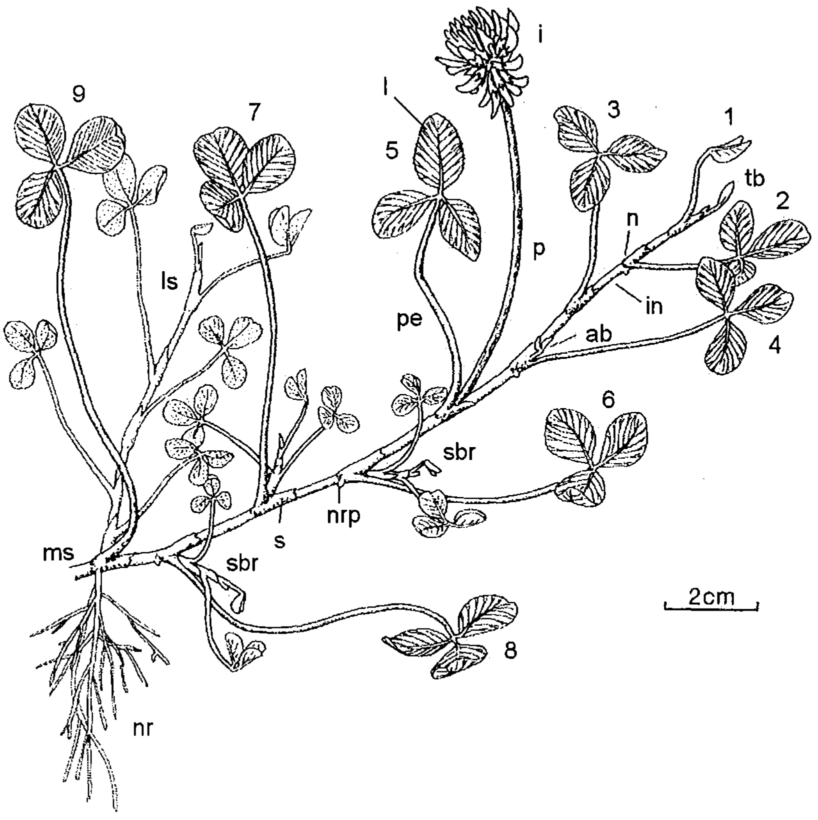

Figure 2.3 Diagram of a main stolon (ms) of white clover. tb = terminal bud, n = node, in = internode, ab = axillary bud, i = inflorescence, p = peduncle, l = leaflet lamina, pe = petiole, sbr = stolon branch, nrp = nodal root primordial, s = stipule, ls = lateral stolon, nr = nodal root. Emerged leaves on the main stolon, and the nodes bearing them, are numbered 1 to 9 (adapted from Thomas (1987a)).

In Caucasian clover, non-flowering branches remain relatively short and slightly ascending to form aerial shoots (Figure 2.2). These develop from buds that have formed above ground in the light. However, buds that are pulled below the soil surface by the seedling contraction develop into underground rhizomes (Figure 2.4). Rhizomes have morphological features similar to stolons, including the presence of nodes, internodes, and nodal root primordia. On rhizomes, leaf primordia develop into small (2–5 mm long) colourless scale leaves rather than the photosynthetic leaves found on stolons. Each scale leaf on a rhizome subtends an axillary bud capable of forming into a rhizome branch, and each node is capable of forming two nodal roots (Jernstedt and Bouton, 1985; Li and Beuselinck, 1996; McKinless and Alderson, 1991; Thomas, 2003). Rhizome growth in Caucasian clover has been reported to be at first totally dependent on carbon from the photosynthetic shoots, and must compete with the leafy shoots and roots for carbon (Thomas, 2003), but this has not been quantified.

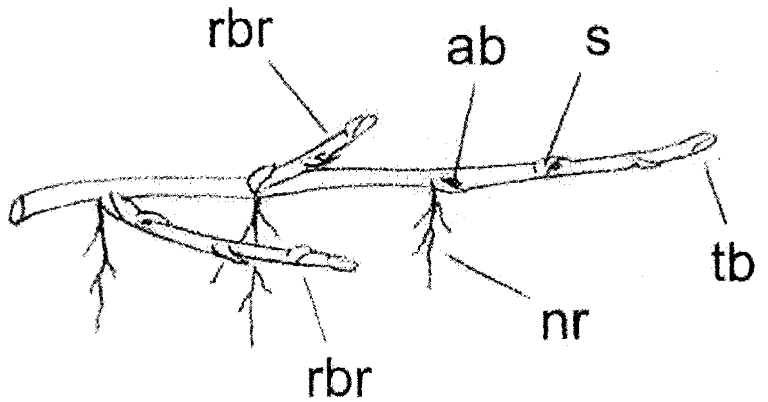

Figure 2.4 Diagram of a rhizome of Caucasian clover. ab = axillary bud, s = scale leaf, tb = terminal bud, nr = nodal root, rbr = branches growing as young rhizomes (Thomas, 2003).

2.2.2.2 *Nodal roots*

Both species are capable of forming nodal roots on a stolon (Figure 2.3) or rhizome (Figure 2.4) under moist conditions. However, the ability to form nodal roots may differ between the two species (Thomas, 2003). In white clover, the nodal root primordium grows into a nodal root when it is in contact with moist soil. Each nodal root most often develops into a fibrous root system rather than a taproot. Nodal roots occur abundantly along the length of the stolon under moist conditions (Thomas, 1987b). Such abundant growth of nodal roots has a strong impact on the allocation of carbon and the subsequent form of the plant. In white clover, carbon allocated to root growth is mostly directed to the nodal root systems along the stolon rather than to the basal root system (Thomas, 2003). This is likely to be a significant factor contributing to the death of the seminal taproot and primary stem axis after about 2 years (Brock *et al.*, 2000; Brock and Tilbrook, 2000).

In Caucasian clover, nodal roots occur frequently along the length of rhizomes under moist conditions (Genrich *et al.*, 1998; Thomas, 2003). Most nodal roots develop into fibrous root systems, but those which develop close to the seminal taproot may develop into secondary taproots (Thomas, 2003). However, in the absence of associated foliage leaves the nodal root system may develop relatively weakly (Thomas, 2003).

2.2.2.3 Taproots

A key additional difference between these two growth forms lies in the ability to form taproots. In white clover, the seminal taproot and primary stem axis rarely survive longer than 18 to 24 months under fertile moist conditions (Brock *et al.*, 2000; Brock and Tilbrook, 2000; Westbrooks and Tesar, 1955). The taproot of white clover has a high proportion of soft parenchymatous tissue, and its early death has been attributed to the invasion of this tissue by pathogens (Westbrooks and Tesar, 1955). Taproot death in white clover may also be hastened by an inadequate supply of carbon from the leaves (Thomas, 2003). This can occur when the carbon supply to taproots is diminished by the presence of competing nodal roots as described in the previous section.

In contrast, a major characteristic of Caucasian clover is its rapid early formation of a deep, semi-woody, often-branching taproot system (Bryant, 1974). In the New Zealand (South Island) high country, Moorhead *et al.* (1994) found that, when strip-seeded into depleted fescue tussock (*Festuca nova zelandiae*) grassland, 5-month-old Caucasian clover plants had taproots deeper than 0.70 m. Also in New Zealand (South Island) high country, Strachan *et al.* (1994) reported that a 13-year-old ungrazed stand of Caucasian clover had more than 20 t DM/ha root biomass (rhizomes plus taproots). In a high altitude site in Australia, Spencer *et al.* (1975) found that a 17-month-old stand had considerably more root mass than aerial parts (2.74 root/shoot ratio cf. 0.16 for white clover). Likewise, Fu *et al.* (2001) reported that 5-month-old Caucasian clover had a root/shoot ratio of 2.52 when grown in a sand bed.

Thus, the root system of Caucasian clover is complicated. It consists of several fractions, described by Peterson *et al.* (1994b) and by Woodman (1999) as the primary crowns, primary taproots, rhizomes, secondary crowns, secondary taproots, fibrous roots, rhizome shoots, and rhizome initials. This complex root system was associated with a large underground biomass which averaged 6600 kg DM/ha under different defoliation treatments in Minnesota, USA (Peterson *et al.*, 1994b). However, the underground biomass of Caucasian clover can be reduced under frequent grazing in mixed pasture (Lucas *et al.*, 1998).

2.2.2.4 Clone formation

Both species are clone formers in that they persist by colonising new areas with vegetatively derived offspring (Beuselinck *et al.*, 1994; Forde *et al.*, 1989). In white clover, the plant spreads and perenniates via stolon growth. Due to the death of the seminal taproot and primary stem axis, these stolons eventually fragment and become independent clone plants which lack taproots. The spreading form of white clover is highly successful in grazed pasture, but is dependent on the development of shallow nodal root systems that are only produced under moist conditions. These smaller plants are more vulnerable to environmental stresses (e.g. temperature, water status, soil fertility, grazing, or pest attack) than the taprooted seedling form (Woodfield and Caradus, 1996). Clonal populations of white clover have been reported to decline 2–3 years after sowing even though initial establishment was satisfactory (Brock and Hay, 2001) which indicates the influence of environmental stresses on growth and persistence of stolon fragments.

In contrast, Caucasian clover spreads and perenniates via rhizome growth. Eventually, after growing below ground for several centimetres, the tip of each below ground rhizome stops elongating and grows upwards into the light where it forms a secondary crown and in many cases develops its own taproot (Genrich *et al.*, 1998; Thomas, 2003). These daughter plants may eventually become independent of the primary crown system, but it is unclear when this occurs. In a montane Australian environment, Dear and Zorin (1985) found 4-year-old Caucasian clover produced 74 daughter plants/parent plant and had rhizomes nearly 500 mm in length. In Minnesota USA, Cuomo *et al.* (2003) reported that in grass mixtures 4-year-old stands of Caucasian clover had spread 1.0 m, and 5-year-old stands had spread 1.5 m, to effectively make the width of the plots 160 and 190% of the initial plot width, respectively.

In summary, the pattern of seedling development through germination to the formation of a rosette seedling is similar for both species. White clover then develops above ground stolons whereas Caucasian clover develops aerial shoots and below ground rhizomes. Both species form nodal roots on their vegetative stems, but the taproots of Caucasian clover are deeper and persist longer than the taproots of white clover. Both species are clone formers in that they spread vegetatively through stolons or rhizomes which produce daughter plants. Thus, these morphological differences underpin the responses of each species to environmental factors in a temperate environment.

2.3 Agronomic responses to environmental factors

The major environmental factors that influence legume productivity in temperate environments are temperature, water status, and soil fertility, while grazing or defoliation is the major management factor (Marten *et al.*, 1989; Scott *et al.*, 1995). The aim of this section is to compare the agronomic responses of white and Caucasian clovers to those environmental factors in temperate environments.

2.3.1 Temperature

Temperature is a major factor affecting legume distribution and persistence (Marten *et al.*, 1989). Temperature varies greatly across regions due to the effects of latitude, topography, altitude, aspect, and coastal proximity. Even within regions of adaptation, legume persistence and productivity are affected by seasonal variations in temperature (McKenzie *et al.*, 1999; Scott *et al.*, 1995). The influence of temperature on white clover has been expressed in terms of seasonal production in grazed perennial ryegrass-dominant pasture at different sites across New Zealand (Radcliffe, 1974). Radcliffe and Baars (1987) compared seasonal growth curves for warmer and cooler sites. They concluded that soil temperature early and late in the growing season was an important limiting factor determining both annual yields and the seasonal pattern of white clover production.

Low temperatures have a major influence on the persistence and productivity of white clover (Eagles and Othman, 1981; Ettema and Ledgard, 1992; Haycock, 1981). The growth of white clover is greatly reduced at temperatures below 18 °C, whereas the growth of perennial ryegrass is less affected between 12 and 18 °C (Mitchell, 1956; Figure 2.5). This intolerance to lower temperatures by white clover is of particular importance during spring, when 10 cm soil temperatures are often between 10 and 18 °C from September to December in New Zealand (Ettema and Ledgard, 1992). As a result, perennial ryegrass is more productive than white clover during this time of year (Brougham, 1959). Furthermore, individual white clover plants are at their smallest in early spring and are further reduced by competition from perennial ryegrass at the lower temperatures (Brock and Hay, 2001). White clover is most productive during summer because of its relatively high optimum temperature of 24 °C compared with 20 °C for perennial ryegrass (Mitchell, 1956; Figure 2.5), but only under moist conditions. In general, white clover is well suited to the maritime humid temperature climate of New Zealand.

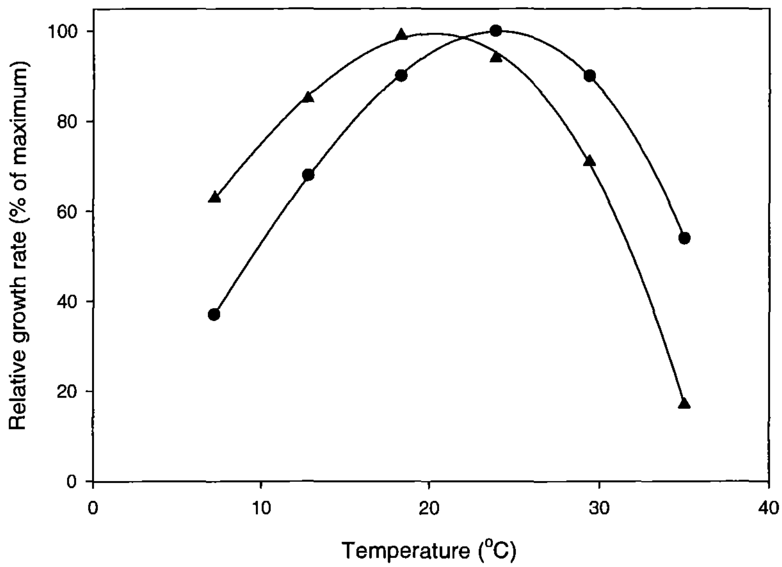

Figure 2.5 Effect of temperature on the relative growth rate of white clover (●) and perennial ryegrass (▲) in controlled environments (Mitchel (1956)).

In contrast, Caucasian clover is a 'winter domant' species, like lucerne (*Medicago sativa*) and chicory (*Cichorium intybus*), which reflects the cold winter temperatures experienced in the continental climates where it evolved (Bryant, 1974). Early research showed the superior adaptation of tetraploid Caucasian clover cultivars over other temperate legume species to high altitude, montane environments in New Zealand and Australia (Dear and Zorin, 1985), and hexaploid cultivars to the long, cold continental winters of the North-Central USA (Zemenchik *et al.*, 2001). Winter survival in extreme continental climates may be attributed to its underground rhizome and root system (Strachan *et al.*, 1994). However, there is no information on the temperature responses of Caucasian clover in more productive lowland conditions where the pattern of seasonal production is important for overall feed supply.

2.3.2 Water status

Water status affects legume distribution and persistence (Marten *et al.*, 1989). Uneven seasonal rainfall, topography, aspect, and water-holding capacity of the soil all contribute to soil water deficits which limit plant growth (McKenzie *et al.*, 1999; Scott *et al.*, 1995). As with temperature, the influence of water status on white clover has often been expressed in terms of seasonal production in grazed pastures at different sites throughout New Zealand

(Radcliffe, 1974). Radcliffe and Baars (1987) compared seasonal growth curves for irrigated and moisture-limited sites and concluded that soil water status during the growing season had a major influence on annual yields and seasonal production patterns. However, the influence of water status was often not expressed in isolation, but in combination with temperature.

Growth of white clover is dependent on the soil water status. Plant populations are susceptible to drought in spring when plant size is at its smallest (Brock *et al.*, 1988), while summer and autumn droughts can cause the death of shallow nodal roots and therefore the collapse of stolon populations (Archer and Robinson, 1989; Gibson and Trautner, 1957; Woodfield and Caradus, 1987). For example, Brock *et al.* (1988) reported that summer drought (rainfall 30% below average) caused white clover content to decrease from 15 to less than 3%, and was associated with a 75 to 90% reduction in stolon DM. Subsequent stolon growing point populations required 2 years to recover to pre-drought levels (Brock and Caradus, 1995). Frequently, the combination of hard summer grazing and soil water deficits exposes the base of the pasture and increases white clover death due to high temperatures (~50 °C) at the soil surface (Archer and Robinson, 1989; Brock and Kim, 1994).

Many of the studies at hill and high country sites have shown that Caucasian clover is more drought tolerant than white clover. In the South Island of New Zealand, Caucasian clover survived better than 18 cultivars and accessions of white clover after 8 years on drought-prone sunny slopes (Woodman *et al.*, 1992). Caucasian clover was also more drought tolerant than white clover on hill country in North Canterbury (Daly and Mason, 1987). In the Snowy Mountains of New South Wales, Australia, Caucasian clover has also been shown to be more persistent than white clover under drought conditions (Dear and Zorin, 1985).

More recent New Zealand research has shown that in lowland regions, Caucasian clover was more productive than white clover under summer drought conditions. Watson *et al.* (1998) compared the seasonal performance of Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures in a year of spring–summer drought in coastal Bay of Plenty. Clovers were sown alone in spring to overcome possible establishment problems, and over drilled with perennial ryegrass in the following winter. Soil moisture dropped as low as 4% volumetric water content (or 9 ml water/250 ml soil) in the top 0.10 m. Growth rates of the 3-year-old pastures peaked around 25 October (65–70 kg DM/ha/d) for CC–RG and around 14 October (50–60 kg DM/ha/d) for WC–RG. As drought intensified the decline in pasture growth rates was similar, but delayed by about 21 d for CC–RG. Both pastures produced less than 10 kg DM/ha/d in mid-summer, but CC–RG had five-times the legume content of WC–RG. The extent of this difference on annual production was not reported.

The performance of white and Caucasian clovers were compared when established with five perennial grass species in Canterbury New Zealand: perennial ryegrass, cocksfoot (*Dactylis glomerata*), tall fescue (*Schedonorus phoenix* syn. *Festuca arundinacea*), grazing brome (*Bromus stamineus*), and phalaris (*Phalaris aquatica*). White clover was more productive than Caucasian clover 15-months after sowing, but dry conditions during years 4 and 5 (~60% of 680 mm mean annual rainfall) decreased the white clover content in all pastures. Rainfall in year 6 was more favourable (111% of mean) when total annual DM production was 10.0 t DM/ha for Caucasian clover pastures compared with 8.7 t DM/ha for white clover pastures. The mean annual white clover content ranged from 9% with perennial ryegrass and phalaris to only 1% with cocksfoot. In contrast, the annual Caucasian clover content averaged 20% across all five pastures, but reached 46% with cocksfoot during summer (Black and Lucas, 2000). Thus, Caucasian clover was more tolerant of summer moisture stress than white clover in mixtures with perennial grass species.

Greater drought tolerance in Caucasian clover has been attributed to its extensive rhizomes and deep taproot. The taproot suggests that Caucasian clover was able to source water from greater depths than white clover, which is dependent on nodal roots after 18–24 months. The rhizomes and underground growing points in Caucasian clover are also protected against high soil surface temperatures and grazing pressure under summer dry conditions. However, there is no information on the water extraction patterns of Caucasian clover, or the physiological basis for its drought tolerance.

2.3.3 Soil fertility

While temperature and water status have a significant influence on legume productivity, they are generally uncontrollable. The third factor, soil fertility, can be most easily modified either by regional or local variations of different natural fertility, or by the addition of fertiliser (McKenzie *et al.*, 1999; Scott *et al.*, 1995).

White clover competes poorly with grasses when soil fertility is limiting. This occurs because the nodal root system of white clover has much less surface area for nutrient uptake (Ettema and Ledgard, 1992; Woodfield and Caradus, 1996). Phosphorus (P) is the major limiting nutrient for white clover growth in New Zealand soils, followed by sulphur (S), potassium (K), and molybdenum (Mo) (During, 1984). White clover becomes a minor component of pastures without regular inputs of these elements. Therefore, most soils in New Zealand require annual maintenance inputs of P and S to ensure adequate white clover growth, while K may be necessary in more intensive grazing systems such as dairying. In contrast, Caucasian clover may be more beneficial in soils where infrequent fertiliser applications are made (Daly and Mason, 1987; Jarvis *et al.*, 1998; Lucas *et al.*, 1981). Probably because early interest in Caucasian clover was in its use as a revegetation species in sub-alpine areas, the impression developed that Caucasian clover is a 'low fertility' species (Scott, 1998).

2.3.3.1 Phosphorus

Some research has shown that Caucasian clover is more productive than white clover on low fertility soils (low pH, P and S availability). One example was on a soil with an Olsen P of 8 μg/ml and a pH of 5.2 in the New Zealand (South Island) high country (Daly and Mason, 1987). Without maintenance superphosphate (9% P, 12% S) 9-year-old Caucasian clover pastures yielded 850 kg DM/ha and had a legume content of 26% compared with 395 kg DM/ha and 11%, respectively, for white clover pastures in a spring harvest. The authors suggested that this advantage was due to Caucasian clover's ability to utilise the natural soil fertility by means of its rhizome mass and deep taproots.

However, Virgona and Dear (1996) showed that the response of Caucasian clover to P applications was greater than for white clover when fertiliser was applied to a low fertility soil (Olsen P 11 μg/ml) in the Snowy Mountains of New South Wales, Australia. Treatments were high fertility (280 kg/ha Mo superphosphate) and low fertility (no fertiliser) after 11 years. Caucasian clover pastures yielded 1960 kg DM/ha and had a legume content of 75% in the high fertility treatment compared with 1040 kg DM/ha and 22%, respectively, in the low fertility treatment. White clover was less productive under both treatments with only 3% legume in the low fertility treatment. However, these results, and those by Daly and Mason (1987), were confounded by short-term moisture stress. Thus, further research is required to separate the responses to these two factors.

In another experiment, Caucasian clover was fertilised with different rates of P at establishment on a low fertility soil (Olsen P 5 µg/ml, pH 5.3) in New Zealand (South Island) high country (Davis, 1991; Strachan *et al.*, 1994). The experiment was not grazed or fertilised after establishment. By year 12, Caucasian clover had become dominant in all plots where ≥100 kg P/ha had been applied at establishment. Maximum production was achieved with 200 kg P/ha, indicating a significant response to P. Phosphorus stored in the underground biomass amounted to 58 kg P/ha in the 50 kg P/ha treatment. Strachan *et al.* (1994) concluded that the ability to store and remobilise nutrients from the rhizomes and roots was a valuable feature of Caucasian clover in high country where maintenance fertiliser applications are infrequent. However, it is important to note this experiment was not grazed and the root biomass would be much reduced under normal grazing (Lucas *et al.*, 1998).

Caucasian clover and white clover were also components of a 25-species mixture sown into a depleted fescue tussock grassland on a low fertility soil in the New Zealand (South Island) high country (Scott, 2001). The mixture was subject to 31 combinations of P and S fertiliser rates and grazed over 19 years. Caucasian clover was slow to establish but increased to become the most dominant legume species in years 10–19 under moderate to high P inputs (50–100 kg P/ha/yr), but only a minor species at lower P inputs (0–20 kg P/ha/yr). In contrast, white clover responded moderately to P fertiliser in years 2–4, but was a minor species in all P treatments in years 8–19. Scott (2001) concluded that Caucasian clover was very persistent and primarily suited to high fertility rather than low fertility soil conditions.

2.3.3.2 Sulphur

Research in New Zealand (South Island) high country has shown that Caucasian clover is similar to white clover in response to S applications (Jarvis *et al.*, 1998). On a sulphur deficient soil (sulphate-S 2 µg/g, Olsen P 6 µg/ml, pH 5.3) at Mesopotamia Station, yield of 4-year-old Caucasian clover increased from 250 kg DM/ha in a zero S treatment to reach a maximum of 1320 kg DM/ha with 40 kg S/ha in a spring harvest. Likewise, Scott (2001) reported that, in years 10–19, the response of Caucasian clover to moderate S inputs (20–50 kg/ha/yr), but the response was greatest when P was also applied. Similarly, in years 2–4, white clover responded moderately to S fertiliser when P was applied, but was a minor species in all S treatments without P, and in all P and S treatments in years 8–19.

2.3.3.3 Nitrogen and nitrogen fixation

Nitrogen is the major element which drives grass/legume production. Legumes obtain most of their N requirements from atmospheric N_2 fixation, and some by direct uptake of soil N and fertiliser N inputs. Grasses are fed N by the legumes, soil N and fertiliser N. Thus, pasture production is dependent on total N availability. Total N yield from a pasture may indicate the N_2 fixation activity by the legume component and the total N cycling within the pasture. This is often calculated from the sum of DM yield and the % N of legumes and grasses:

Equation 2.1 Total N in legume (kg/ha) = DM of legume (kg/ha) × %N in legume/100

and

Equation 2.2 Total N in grass (kg/ha) = DM of grass (kg/ha) × %N in grass/100

N_2 fixation can be measured using ^{15}N, labelled ammonium sulphate (40 atom% ^{15}N) watered onto a pasture (Ledgard *et al.*, 1985). The ^{15}N is a non-radioactive isotope of N and can be measured using a mass spectrometer, which can also measure the naturally occurring ^{14}N. A first approximation is that the grass takes up the inorganic ^{15}N from the soil, while in the legume, the uptake of labelled ^{15}N is diluted by the N_2 fixation (the fixed N contains largely only ^{14}N from the atmospheric N_2 gas). The difference in uptake of ^{15}N and ^{14}N enables the proportion of N fixed by the legumes (P_N) to be calculated as:

Equation 2.3 $P_N (\%) = 100 \times (G - L)/(G - B)$

where G and L are the ^{15}N concentrations of grass and legume, respectively. B is the ^{15}N concentration of legume completely dependent on atmospheric N_2 and a value of 0.3663 atom% ^{15}N was used in all calculations (Ledgard, 1989). The amount of fixed N (kg/ha) in the cut legume herbage can then be calculated as:

Equation 2.4 Fixed N (kg/ha) = DM of legume (kg/ha) × %N in legume/100 × P_N/100

2.3.3.3.1 <u>Inoculation</u>

Both species differ in their inoculation requirements for N_2 fixation. Root-nodule bacteria that form nodules and fix N_2 for white clover are present in most New Zealand pastoral soils and therefore inoculation is not necessary for nodulation when resowing paddocks with this species. However, root-nodule bacteria may be absent, or present in small populations, in some less developed areas (Pryor *et al.*, 1998).

In contrast, root-nodule bacteria that form nodules on Caucasian clover are absent from New Zealand soils and therefore inoculation with the correct strain of root-nodule bacteria is essential for N_2 fixation and for establishment. The performance of Caucasian clover improved after the selection of the *Rhizobium* strain ICC148, which is specific to hexaploid cultivars. Inoculation with this strain resulted in improved establishment (Section 2.5.3), more effective nodulation and greater N_2 fixation, particularly in tussock grasslands (Pryor *et al.*, 1998).

2.3.3.3.2 <u>N_2 fixation</u>

Biological N_2 fixation by legumes is both ecologically and economically vital to pastoral farming in New Zealand (Walker, 1995). The N yield and N_2 fixation ability of white clover has been studied extensively in grazed pastures at different sites throughout New Zealand (Ball *et al.*, 1979). Hoglund *et al.* (1979) compared seasonal N yield and N_2 fixation rates for warmer and cooler, irrigated and moisture-limited, well-fertilised (P, K and S) and nutrient-limited sites. For example, in Canterbury, irrigated and dryland pastures fixed 190 and 120 kg N/ha/year, respectively. Hoglund *et al.* (1979) concluded that clover DM yield was closely related to clover N yield but not necessarily related to N_2 fixation rate, due to direct uptake of soil N. However, a common view in the literature is that New Zealand's white clover-based pastures are fixing insufficient N to support the demands of grasses with a high production potential for the provision of enough quality feed for maximum animal production (Caradus *et al.*, 1996; Chapman *et al.*, 1995; Clark *et al.*, 1995).

Few experiments have reported the N_2 fixation of established Caucasian clover. In Minnesota USA, N_2 fixation by 2–3-year-old stands of Caucasian clover and birdsfoot trefoil (*Lotus corniculatus* L.) was determined using the ^{15}N method. Stands were cut four times per year. Annual total DM, total N, and fixed N yields of Caucasian clover averaged 8.85 t DM/ha, 271 kg N/ha, and 155 kg N_2/ha, respectively, compared with 8.00 t DM/ha, 233 kg N/ha, and 147 kg N_2/ha, respectively, for birdsfoot trefoil. The proportion of N derived from N_2 fixation (P_N) averaged 57% for Caucasian clover and 62% for birdsfoot trefoil, and was stable across harvests for Caucasian clover (Seguin *et al.*, 2000).

In Wisconsin USA, fertiliser N replacement values (FNRVs) of Caucasian clover and birdsfoot trefoil were compared when grown in mixtures with three cool-season grass species: Kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* Leyss.), or cocksfoot. The FNRV is the amount of N fertiliser required for a grass monoculture to yield as much DM as the same grass grown in mixture with a legume. The 1–3-year-old mixtures were cut three times per year. The FNRV for both legumes correlated positively with total season legume yield. Mean annual FNRVs ranged from 74 to 325 kg N/ha for Caucasian clover and from 147 to 295 kg N/ha for birdsfoot trefoil (Zemenchik *et al.*, 2001).

Because of the differences in N dynamics between ungrazed and grazed mixtures, additional data are needed to determine the N yield and N_2 fixation by Caucasian clover in intensively grazed pastures. Such a study was conducted in Canterbury New Zealand, and interim results showed that Caucasian clover fixed more N than white clover in irrigated ryegrass pastures (Widdup *et al.*, 2001). Additional results from this study are presented in Chapter 4.

2.3.3.4 Soil pH

Caucasian clover is more persistent than white clover on acid soils (Bryant, 1974; Daly and Mason, 1987; Scott, 2001), but like white clover it also responds to lime (Barnard and Folscher, 1988). On a soil with a pH of 4.65, DM yields of both Caucasian and white clovers increased with liming which raised the pH to 6.0. Foliar analyses indicated that poor growth on acid soils could largely be attributed to inadequate Ca and Mo uptake. In glasshouse pot experiments, increased yields of Caucasian clover seedlings in response to liming was explained by alleviation of Al, Zn and Mn toxicities and increased availability of P and Mo at high soil pH levels. Caucasian clover was also consistently more responsive to increased soil pH than birdsfoot trefoil (DeHaan *et al.*, 2002). In New Zealand, Caucasian clover has shown greater tolerance than white clover, but similar tolerance to Maku lotus (*Lotus pedunculatus*), to elevated soil Al levels in tussock grassland sites (Caradus *et al.*, 2001).

2.3.4 Grazing management

The persistence and long-term productivity of legumes in pastures is influenced by their response to grazing or defoliation (Sheath and Hodgson, 1989). Some of the long-lived stands of Caucasian clover have persisted under very lax grazing, if grazed at all (e.g.

Strachan *et al.*, 1994). Persistence of Caucasian clover under grazing is important if it is to complement white clover in temperate pastures. In one study, Caucasian clover and 12 cultivars and accessions of white clover, transplanted to overcome possible establishment problems, were subjected to nine grazing regimes in New Zealand (South Island) high country: low, medium and high stocking rates with continuous, alternating, or rotational grazing. After 5 years none of the white clover cultivars survived better than Caucasian clover. Spread of Caucasian clover transplants after 9 years averaged 582 mm under continuous, alternating and rotational grazing, but was greater under medium and high than under low stocking rates (Allan and Keoghan, 1994).

Scott (2001) subjected Caucasian and white clovers, in a 31-species mixture (including eight sown legumes and seven sown grasses), to 19 years of different grazing and fertiliser managements in New Zealand (South Island) high country: low, medium and high stocking rates with continuous or rotational grazing. Caucasian clover was slow to establish but increased over time to become a major species in many of the treatment combinations. The treatment effects on Caucasian clover were small, with greater abundance under low stocking rate treatments in years 2–7, but greater abundance in the moderate and high stocking rate than continuously grazed treatments. Grazing management initially had a small effect on white clover's abundance, but later, abundance increased under continuous grazing at medium and high stocking rates.

Most of the studies on Caucasian clover defoliation from fertile lowland sites have described legume-dominant stands under lax rotational grazing or cutting. In Canterbury NZ, tetraploid Caucasian clover yielded about 20% less under monthly cutting than under bi-monthly cuts (Stewart and Daly, 1980). In Minnesota USA, under 2, 3, or 4 cuts annually, Caucasian clover yielded less than other legumes such as lucerne, red clover (*T. pratense* L.), and birdsfoot trefoil in the first 2 years of production, but subsequently out-yielded red clover and birdsfoot trefoil in all cutting treatments (Sheaffer and Marten, 1991). Also in Minnesota, Caucasian clover was an aggressive competitor against birdsfoot trefoil under lenient rotational grazing by sheep (Sheaffer *et al.*, 1992).

Research in Minnesota continued with 5-year-old stands of Caucasian clover subjected to 3, 4, 5, or 6 cuts annually, and under continuous, 14-, or 28-d rotational grazing by sheep (Peterson *et al.*, 1994a). In the third year of the study, the 6-cut treatment yielded 30% less DM than the

3-cut treatment. In the second and third years, the 14-d rotation treatments produced 28 and 16% less DM, respectively, than the 28-d rotation treatments, but the lowest yields were under continuous grazing. Belowground productivity was also examined (Peterson *et al.*, 1994b). Cutting treatments had little effect on below-ground morphology, or total non-structural carbohydrate (TNC) concentrations. The TNC concentrations were lowest in spring and greatest in autumn, similar to other tap rooted perennial legumes such as lucerne (Moot *et al.*, 2003). Peterson *et al.* (1994b) concluded that the extensive crown-rhizome-root system of established Caucasian clover conferred the ability to maintain adequate TNC concentrations for persistence under a range of defoliation regimes.

Few experiments have challenged Caucasian clover with frequent, intensive grazing in a mixture with an aggressive grass species. Such a study, in Canterbury New Zealand (Lucas *et al.*, 1998), compared the responses of Caucasian clover to different grazing regimes with those of white clover in an endophyte (*Neotyphodium lolii*)-infected hybrid ryegrass (*Lolium* sp.) pasture. After 2 years, continuous grazing by sheep reduced Caucasian clover cover to 10% compared with 25.5% in 25-d rotational grazing treatments. Also, continuous grazing reduced Caucasian clover rhizome plus root DM to 780 kg/ha (to 100 mm depth) compared with 3220 kg/ha in 25-d rotational grazing treatments. White clover cover averaged 21% under continuous grazing and 14-26% under rotational grazing. The authors surmised that persistence of Caucasian clover might be compromised under this extreme continuous grazing management.

2.4 Utilisation

2.4.1 Nutritive value

2.4.1.1 Digestibility

Most of the studies on Caucasian clover nutritive value have been carried out under lax rotational grazing or clipping, under fertile moist conditions in the USA. In Connecticut, *in vitro* dry matter digestibility (DMD) of Caucasian clover averaged 70–82% compared with 67–75% for white clover (Allinson *et al.*, 1985). In Minnesota, *in vitro* DMD of Caucasian clover (67–75%) was higher than other forage legumes such as lucerne, red clover, and birdsfoot trefoil (Sheaffer and Marten, 1991). In other USA experiments, *in vitro* DMD values for Caucasian clover (75–88%) and its grass mixtures (68–79%) consistently exceeded those for other forage legumes (Peterson *et al.*, 1994a; Sheaffer *et al.*, 1992; Sleugh *et al.*, 2000).

Few experiments have reported the nutritive value of Caucasian clover growing under extensive field conditions. In the Snowy Mountains of New South Wales, Australia, *in vitro* organic matter digestibility of Caucasian clover–grass averaged 72% compared with 67% for white clover–grass after 11 years under low-input (P, S and Mo fertiliser) conditions (Virgona and Dear, 1996). This difference was attributed to Caucasian clover's superior persistence and therefore greater legume content in this environment (Section 2.3.3.1). In Minnesota USA, Caucasian clover had greater *in vitro* DMD values compared with lucerne and red clover under soil moisture deficit (Seguin *et al.*, 2002).

2.4.1.2 Crude protein

Crude protein (CP) values reported by Allinson *et al.* (1985) for Caucasian clover were 18–22% compared with 23–25% for white clover. Other experiments under fertile moist conditions in the USA have shown that CP values for Caucasian clover (17–25%) and its grass mixtures (13–25%) consistently exceeded those for other forage legumes such as lucerne, red clover and birdsfoot trefoil (Mourino *et al.*, 2003; Peterson *et al.*, 1994a; Sheaffer *et al.*, 1992; Sleugh *et al.*, 2000).

In the Snowy Mountains of New South Wales, Australia, CP values obtained by Virgona and Dear (1996) after 11 years under low-input conditions were 11% for Caucasian clover–grass compared with 9% for white clover–grass, highlighting the superior persistence of Caucasian clover in this environment (Section 2.3.3.1). In Minnesota USA, Seguin *et al.* (2002) found similar CP values for Caucasian clover, lucerne and red clover under water stress.

2.4.1.3 Minerals

Mineral (Ca, Mg, K, P, Fe, Mn, Cu, Zn) concentrations obtained by Allinson *et al.* (1985) for Caucasian clover were similar to those for white clover. In New Zealand, herbage Na concentrations for white clover averaged 0.31 and 0.05% in coastal lowland and central high country sites, respectively, compared with 0.02% for Caucasian clover in both environments

(Jarvis *et al.*, 1998). Caucasian clover is therefore a low Na (natrophobe) species (Smith *et al.*, 1978), like lucerne, red clover and timothy (*Phleum pratense*) (Edmeades and O'Connor, 2003; Grace, 1983), and white clover is a high Na (natrophile) species like perennial ryegrass.

2.4.1.4 Acceptability

The acceptability of Caucasian clover has been shown to be similar to white clover in a "cafeteria style" (*ad libitum* feeding) grazing experiment in New South Wales, Australia (Hill *et al.*, 1995). Sheep grazed more readily the leaves of both species that were easy to harvest, i.e., the large-leafed tall types.

2.4.1.5 Average daily liveweight gain and liveweight gain per hectare

In Minnesota USA, lamb liveweight gains during 4 years of lax rotational grazing averaged 198, 190 and 205 g/head/d and 705, 860 and 878 kg/ha for birdsfoot trefoil, birdsfoot trefoil–Caucasian clover mixture and Caucasian clover, respectively. The average annual grazing period was 98 d (Sheaffer *et al.*, 1992). In Wisconsin USA, Holstein steers grazing Caucasian clover–grass mixtures gained an average of 1.21 kg/head/d and 1021 kg/ha compared with 0.99 kg/head/d and 800 kg/ha for those on red clover–grass mixtures during 3 years of rotational grazing. The average annual grazing period was 161 d. This advantage of Caucasian clover–grass was attributed to the mixture's superior productivity and nutritive value. Both were consequences of the ability of Caucasian clover to maintain a greater proportion of legume in the pasture (average 68%) compared with red clover (average 21%), which was "frost-seeded" every March over the 3-year period (Mourino *et al.*, 2003).

2.4.2 Anti-quality components

Ruminant bloat is a problem in pastures containing a high proportion of Caucasian clover. This is similar in occurrence to other high quality forage legumes, but can be controlled remedially with poloxalene (Mourino *et al.*, 2003; Sheaffer *et al.*, 1992). Caucasian clover herbage contains non-lethal amounts of hydrocyanic acid (Hill *et al.*, 1995) and no tannins (Broderick and Albrecht, 1997), which is similar to white clover.

2.5 Establishment

In summary, the data reviewed in Sections 2.2–2.4 show that Caucasian clover is adapted to a wider range of environments than white clover, and can make an equal or greater contribution to N inputs and animal performance than white clover. However, a key feature to attaining this performance is ensuring an adequate plant population of Caucasian clover is achieved at establishment.

The major deficiency of Caucasian clover is slow establishment. Thus, a considerable amount of research has been conducted to determine the plant and environmental factors contributing to this problem. Several components of establishment are common to both species, while differences usually highlight the deficiencies of Caucasian clover. Consequently, efforts have been made to devise the most efficient establishment methods.

2.5.1 Soil fertility

Both species responded to increasing rates of P at establishment when oversown into low fertility soil (pH of 4.9–5.5, Olsen P of 3–10 μg/ml) corrected for S and Mo on a tussock grassland site at Mesopotamia Station, Canterbury New Zealand (Lucas *et al.*, 1981). On another low fertility site (pH 5.2, Olsen P 6 μg/ml, sulphate-S 2 μg/g) at Mesopotamia Station, root weight of 5-month-old Caucasian clover plants increased from 0.528 to 0.857 g/plant (to 0.15 m depth) when the rate of molybdic sulphur superphosphate (8% P, 20% S) was increased from 150 to 300 kg/ha, and when the fertiliser was placed beneath the seed using a strip-seeding method rather than with the seed in sod seeding and broadcast methods (Moorhead *et al.*, 1994). It can be concluded that where necessary, remedial fertiliser applications are essential to improve the establishment of both legume species.

2.5.2 Germination

Germination, as a percentage of Caucasian clover seed oversown, was similar to that of white clover over a range of tussock grassland sites (Lowther and Patrick, 1992). In another experiment, seeds of Caucasian clover germinated well under warm–dry conditions but not as adequately under cool–dry conditions on hill country sites (Awan *et al.*, 1993). However, glasshouse pot experiments have indicated that germination of Caucasian clover may be poorer than white clover under marginal conditions owing to slower germination (Awan *et al.*, 1996) and difficulty of the larger diameter radicle entering the soil (Toddhunter, 1997).

In growth chambers, seeds of white and Caucasian clovers showed similar reductions in germination percentage and increases in time to 50% germination at day/night temperatures of 12/6 and 8/2 °C, but not at 24/20, 20/15, and 15/10 °C (Hill and Luck, 1991). The authors concluded that the germination of Caucasian clover is unlikely to be a problem for its wider use in pastures.

2.5.3 Seedling growth and development

In growth chambers, the rate of cotyledon expansion and area of cotyledons 7 d after seeding was greater for Caucasian clover than white clover at day/night temperatures of 15/10, 20/15, and 24/20 °C. However, 34 d after seeding, the number of Caucasian clover leaves per plant, leaf area, and relative growth rates were less than white clover, but shoot and root dry weights were similar (Hill and Luck, 1991). In lowland conditions, Watson *et al.* (1996) reported that the rate of Caucasian clover growing point development (number/m^2) was less than half the rate of white clover in the establishment year.

Slow seedling growth and development in Caucasian clover have been attributed to substantial allocation of energy resources to roots and rhizomes (Taylor and Smith, 1998). In tussock grassland, growth of Caucasian clover in the establishment year was mostly confined to primary crown and taproot/fibrous root growth (Woodman, 1999). In lowland conditions, the number of seedlings emerged after 7, 14, and 21 d was similar for Caucasian and white clovers. However, 65 d after seeding, Caucasian clover partitioned 1:1 shoot to root dry weight compared with 3:1 for white clover, and Caucasian clover seedlings had similar shoot growth but three times the root growth of white clover. Between 65 and 165 d, Caucasian clover partitioned more DM to root and rhizome growth, resulting in a 0.3:1 shoot/root ratio compared with 2:1 for white clover (Widdup *et al.*, 1998). Likewise, in Minnesota USA, Caucasian clover had shoot/root ratios of 0.3–0.5 after 1 year (Genrich *et al.*, 1998).

The slow initial shoot growth and development in Caucasian clover results in lower dry matter yield than white clover in the establishment year. In Canterbury New Zealand, 10 months after sowing, white clover–ryegrass pastures had a legume content of 20% compared with only 4% for Caucasian clover–ryegrass pastures. However, over the following 18 months, the proportion of white clover declined and the proportion of Caucasian clover increased, resulting in Caucasian clover–ryegrass pastures that had a legume content of 28% compared with 10% for white clover–ryegrass pastures after 28 months (Moss *et al.*, 1996).

Recent research has suggested selection for increased shoot/root ratio in Caucasian clover seedlings may be a means of increasing DM partitioning to the shoot, thereby increasing the rate of establishment (DeHaan *et al.*, 2001; Widdup *et al.*, 1998). In both cases, genetic potential for increased shoot growth was found. However, the physiological basis for slow shoot growth and development in Caucasian clover has not been fully explored.

2.5.4 Nodulation/inoculation

Effective nodulation has been identified as a major factor influencing the establishment of Caucasian clover in oversown tussock grassland (Lowther and Patrick, 1992). More effective nodulation of Caucasian clover resulted from increasing the peat inoculant level from the recommended rate (9.6 g peat inoculant/kg seed; 23 000 rhizobia/seed at sowing) to 6.3 times the rate (Patrick *et al.*, 1994). Number of established plants of Caucasian clover was strongly correlated with percentage nodulation 7 months after sowing on seven of nine sites (Patrick and Lowther, 1995).

More recently the nodulation and growth of hexaploid Caucasian clover cultivars has been improved with the commercial release of the superior *Rhizobium* strain ICC148 (Pryor *et al.*, 1998). However, even with this new strain of rhizobia, high numbers of rhizobia on the seed are still required for drilling, and in particular oversowing (Lowther *et al.*, 1998).

2.5.5 Grass competition and seeding rate

In a glasshouse pot experiment, Caucasian clover seedlings were more severely affected by competition from tall fescue than either white clover or birdsfoot trefoil. Roots of Caucasian clover did not branch and spread among the root mass of tall fescue in the same manner as white clover (Hill and Hoveland, 1993). The authors concluded that Caucasian clover could be very sensitive to the seeding rate of companion grass species, particularly on shallow soils with restricted rooting depth.

Further work used larger pots (390 mm wide and 330 mm deep), but excluded white clover and included two soil fertility (N) treatments. Rhizome numbers, and root and rhizome biomass were reduced by increasing grass seeding rates, and at high soil N where grass competition was vigorous compared with low soil N where grass competition was weak (Hill and Mulcahy, 1995). The authors concluded that early growth of Caucasian clover, particularly root and rhizome development, will be improved where the seeding rate of the companion grass is low or grass vigour is low due to inadequate nitrogen. They suggested that Caucasian clover should be established as a pure stand with a companion grass being introduced later.

In New Zealand, most pastures are sown with commercially recommended rates of 16–20 kg/ha of perennial ryegrass (Charlton, 1991; Sangakkara *et al.*, 1982). These rates are primarily used to achieve rapid early production and to suppress weeds, but slower-establishing sown species are also adversely affected. Cullen (1958) showed that slow-establishing pasture species (e.g. white clover, timothy, and cocksfoot) benefit from perennial ryegrass seeding rates of less than 10 kg/ha. In Canterbury New Zealand, Dumbleton (1997) showed there was no significant advantage, in either total yield or weed suppression, from sowing more than 12 kg/ha of perennial ryegrass; a pasture containing more than 20% white clover/chicory was obtained only by sowing in late summer (4 February) with less than 8 kg/ha of ryegrass. Reduction in ryegrass seeding rate reduced inter-plant competition for limited resources, particularly light (Brougham, 1953). In Ireland, Culleton (1986) found no significant differences in total annual yield during the first 3 years from sowing 10 to 35 kg/ha of perennial ryegrass.

2.5.6 Time of sowing

There is limited recent published information on the influence of sowing time. In theory, the optimal time of sowing for Caucasian clover is similar to white clover in that it is primarily related to the avoidance of climatic constraints that inhibit its establishment. Frame and Newbould (1986) provided an agronomic outline of the time of sowing requirements for white clover. Specifically, mid-summer is often associated with soil moisture deficits. Late-autumn sowing allows insufficient time for adequate plant development before the onset of low temperatures in winter. For example, a late-autumn sowing in Canterbury New Zealand, when soil temperature was below 13 °C, resulted in perennial ryegrass dominance, increased weed content, and no white clover or chicory in the pasture mixture (Dumbleton, 1997). Mortality of autumn-sown clover can be hastened due to attack by *Pythium* and other fungi which cause "damping off" of seedlings. Spring is therefore the best time for sowing perennial legumes in temperate environments. Very early spring sowing could also have low-temperature limitations on legume establishment (Frame and Newbould, 1986).

In practice, several factors including farm operations and local weather patterns will influence the time of sowing. For example, historically, the majority (83%) of pastures in New Zealand are autumn sown (Sangakkara *et al.*, 1982). In theory, spring sowing offers the best chance for Caucasian clover establishment since there is time for manipulation of grazing management, weed control, and other measures to encourage its establishment. For example, sowing Caucasian clover as a pure species in spring and over-drilling ryegrass in the following winter (July) was a successful and cost effective method for establishing this species in warm temperate coastal Bay of Plenty New Zealand (Taylor and Watson, 1998; Watson *et al.*, 1996).

2.5.7 Sowing method

Experiments have been carried out to determine the most effective method for establishing Caucasian clover. Strip-seeding, sod-seeding, and broadcast-sowing methods were evaluated for introducing Caucasian clover into a depleted fescue tussock grassland site at Mesopotamia Station, Canterbury New Zealand. The strip-seeding method cut and inverted a ribbon of turf, placing it adjacent to the drilling strip; fertiliser was banded at ~50 mm depth; a seed coulter sowed the seed (~10 mm depth), and a press wheel firmed the seed bed. The sod-seeding method used an inverted T coulter, with fertiliser placed with the seed. The broadcast method involved seeds and fertiliser sown together onto a 200-mm wide strip of resident vegetation. After 2.5 months, 48 and 38% establishment had occurred in the strip and sod-seeding, respectively, but only 9% from the broadcast-seeding. Strip-seeding was the most successful method, resulting in earlier rhizome and taproot development, and wider lateral spread of rhizomes. Both strip- and sod-seeding resulted in all plants developing rhizomes 9 months after sowing, but plants from broadcasting were small with very few rhizomes. Removal of competing vegetation was critical in achieving rapid establishment (Moorhead *et al.*, 1994).

Four sowing strategies were evaluated for establishing Caucasian, white and red clovers on an irrigated dairy farm in lowland North Otago New Zealand (Hurst *et al.*, 2000). These were: 1) temporal separation of species (clovers sown in spring before perennial ryegrass undersown at 10 kg/ha in autumn); 2) substitution of ryegrass with slower-establishing timothy; 3) physical separation (alternate 75-mm-spaced drill rows) of slower-establishing species (Caucasian and white clovers, and timothy) from perennial ryegrass and red clover; 4) use of lower than average ryegrass seeding rates of 3.5 or 8 kg/ha. After 16 months, high yielding and high quality pastures were established using all four sowing methods. Legume content was similar in all treatments but the proportion of legume was much lower for Caucasian (1%) than white (37%) and red (17%) clovers. Mean proportion of seeds sown established was lower for Caucasian (48%) than white (74%) and red (74%) clovers 43 d after sowing. This on-farm study demonstrated successful establishment of red and white clovers in all four treatments, but Caucasian clover was suppressed by the inclusion of low rates of ryegrass, red and white clovers, and timothy in the mixture.

2.6 Plant growth and development responses to temperature and water status

For white and Caucasian clovers in non-limiting soil fertility conditions, the main environmental factors influencing growth have been shown to be temperature and water status (Sections 2.3.1 and 2.3.2). The influence of these factors on white clover has been studied intensively. Specifically, temperature and water status affect both the rate of plant growth (e.g. shoot growth, photosynthesis) and the rate of developmental stages (e.g. germination, emergence, leaf appearance, and branching) and therefore yield of white clover. Also, the response of white clover to water status has been researched with regard to its water use and water use efficiency (WUE). In contrast, the influence of temperature and water status on Caucasian clover has usually been expressed in terms of seasonal production (Section 2.3.2), without any explanation of the mechanisms responsible for the responses. Thus, an understanding of the plant growth and development responses to temperature and water status is necessary to understand observed differences in productivity and establishment between the two species.

2.6.1 Temperature

Temperature affects the rate of plant growth and development. The rate of plant processes such as photosynthesis (an indicator of potential growth), nutrient uptake, and N_2 fixation are also dependent on temperature. In controlled environments, Mitchell (1956) and Mitchell and Lucanus (1962) reported that shoot growth of white clover increased with temperature up to an optimum of ~24 °C before it declined (Figure 2.5).

2.6.1.1 Temperature and photosynthesis

Under non-limiting light conditions, the rate of leaf photosynthesis of temperate pasture species increases with temperature up to an optimum (Charles-Edwards *et al.*, 1971; Peri *et al.*, 2002b). In controlled environments, the rate of photosynthesis of leaves of white clover grown at day/night temperature of 18/14 °C was measured at a range of leaf temperatures (3, 8, 13, 18, and 23 °C) at a light intensity of ~250 $J/m^2/s$. There was a large effect of temperature: net photosynthesis rate of youngest expanded leaves initially grown at 18/14 °C was 7.6 μmol $CO_2/m^2/s$ when measured at 3 °C, and photosynthetic rate increased linearly by an average of 0.85 μmol $CO_2/m^2/s$ per °C to 24.6 μmol $CO_2/m^2/s$ at 23 °C. The response of leaf photosynthesis to measurement temperature was less under low irradiance (~90 and ~50 $J/m^2/s$) (Woledge and Dennis, 1982).

However, photosynthesis rates of white clover appeared to be less sensitive to high temperatures than low temperatures. Blaikie (1988b) found that, at a high light intensity (~2000 μmol/m^2/s photosynthetic photon flux density (PPFD)), temperatures between 24 and 33 °C had little effect on the rate of canopy photosynthesis (30.6–37.5 μmol $CO_2/m^2/s$) of well-fertilised, well-watered white clover pastures. Maximum rates of canopy photosynthesis occurred at 29.1–31.0 °C, ~6 °C higher than the optimum temperature for shoot growth (24 °C) in controlled environments reported by Mitchell (1956) and Mitchell and Lucanus (1962). Rates of Caucasian clover photosynthesis in response to temperature and water status have not been reported.

2.6.1.2 Temperature and plant development

Temperate also affects the rate of leaf appearance of plants. In growth chambers, Mitchell and Lucanus (1960) found that the number of days between the appearance of successive leaves on a stolon apex of white clover was 14.3 d at day/night temperatures of 7.2/1.7 °C but decreased as temperature increased to 6.7 d at 15.6/7.2 °C. Haycock (1981) reported that the rate of leaf appearance per stolon apex in white clover grown at a constant 4.8, 6.3, 8.6, or 11.5 °C was ~0.20, 0.35, 0.50 and 0.82 /week, respectively. A line fitted to this linear relationship indicated a base temperature for leaf appearance at 2.6 °C. However, leaves produced remained small until temperatures exceeded 6.3 °C.

Temperature also affects branch development of plants. In growth chambers, Mitchell (1956) found that as temperature increased from 7.2 to 35.0 °C under a 12-h day length, rate of stolon branching in white clover increased to a maximum at 21 °C and then declined. These results were confirmed by Mitchell and Lucanus (1962) who repeated the experiment under a 16-h day length. However, in both cases the response was small (4–7% increase) compared with shoot growth (5–15% increase), or leaf appearance rate by Haycock (1981). Thus, leaf appearance rate on a stem apex and leaf photosynthesis rate are primary components of legume production that are strongly regulated by temperature.

2.6.1.3 Thermal time

Because temperature affects the rate of plant development, this relationship is often quantified using thermal time, or growing degree days (°Cd) (Arnold and Monteith, 1974). Thermal time is the cumulative temperature above a species-dependent base temperature below which development ceases. It is often calculated on a daily basis using Equation 2.5.

Equation 2.5

$$\textit{Thermal time } (Tt \textit{ in } {}^{\circ}Cd) = \sum\left(\frac{T\max + T\min}{2}\right) - T_b$$

where Tmax and Tmin are the daily maximum and minimum temperatures, and T_b is the base temperature.

If development rate is related to temperature then the duration between two developmental stages will be a function of the mean temperature and the duration of the period (Charles-Edwards *et al.*, 1986). Thus, the thermal time requirement and T_b can be determined for any developmental stage (e.g. germination, emergence, leaf appearance, or branch development) using a linear regression technique (Goudriaan and van Laar, 1994). Specifically, a plot of mean temperature and days taken between two developmental stages gives a relationship that decreases exponentially to the optimum temperature (T_{opt}), where development rate is greatest. By plotting development rate (1/d) against mean temperature below T_{opt}, a linear relationship is obtained from which T_b and the thermal time requirement can be determined.

Thermal time has been widely used in annual crop research (e.g. Jamieson *et al.*, 1998), but has also been used to quantify development in temperate pasture species (e.g. Angus *et al.*, 1981; Hsu and Nelson, 1986a). Moot *et al.* (2000) used thermal time to quantify differences in germination and seedling emergence of several temperate pasture species such as white clover and perennial ryegrass, but did not include Caucasian clover. White clover, which had a T_b of 0 °C, required 45 °Cd to germinate (75% germination) and 150 °Cd to emerge (50% emergence) after sowing at a depth of 20 mm. These results are equivalent to 5 and 15 d, respectively, if the mean temperature was 10 °C. In comparison, perennial ryegrass, with a T_b of 2.0 °C, required 90 °Cd to germinate but 160 °Cd to emerge, or 9 and 16 d, respectively, at a mean temperature of 10 °C. Thus, species differ in their response to temperature due to differences in both the T_b and thermal time requirements for development.

While a number of studies have measured the developmental responses of white clover to temperature, few have quantified them using thermal time. For example, Haycock (1981) found that the rate of leaf appearance per stolon apex in white clover was linearly related to temperature from 4.8 to 11.5 °C, and calculated a threshold or T_b of 2.6 °C. However, the leaf appearance interval (1/leaf appearance rate), or phyllochron (phyllo = leaf, chron = time) for successive leaves on the stolon apex was not reported. Reanalysis of the data from Haycock (1981) showed that the phyllochron differed in days (exponentially from ~35 d at 4.8 °C to ~8.8 d at 11.5 °C) but was constant in thermal time (~79 °Cd). Similarly, Arnott and Ryle (1982) reported the leaf appearance interval of white clover (in days) at different temperatures, which when reanalysed gave a phyllochron of ~70 °Cd. Hill and Luck (1991) reported a T_b temperature for germination of 5.24 °C for Caucasian clover and 5.79 °C for white clover, but thermal time requirements for germination were not reported.

The thermal time approach has some limitations. For most plant species the rate of plant development is not linearly related to temperature. In reality the relationship is more likely to be curvilinear with a significant tail at temperatures approaching T_b (Angus *et al.*, 1981; Hsu *et al.*, 1984). This has two important implications. Firstly the physiological and calculated base temperatures are unlikely to coincide; at the calculated value of T_b there is still likely to be some development occurring (Arnold, 1959). However, this error is likely to be small and of little practical significance. The second implication is that because the T_b is extrapolated the calculated value will depend on the range of temperatures used. Thus, more data from the entire sub-optimum (i.e. $\leq T_{opt}$) thermal range will give better estimates of T_b and thermal time requirements.

Cardinal temperatures quantify the range of temperatures that contributes to plant development. Both T_b and T_{opt} have been defined. A third cardinal, the maximum temperature (T_{max}), defines the upper threshold where no development takes place. In most temperate climates plants are exposed to temperatures between T_b and T_{opt}. Thus, the use of a positive linear response at sub-optimum temperatures is appropriate and gives a reasonable estimate of T_b. When T_{opt} has been exceeded results should be excluded from the linear determination of thermal time and T_b, but can be used to give an indication of T_{opt}.

2.6.2 Water status

Actively growing pasture plants are usually 80–90% water. The main function of water in a plant is structural support of cells (cell turgor) and therefore the plant. Water flows through the plants from the soil to the air in the transpiration flow. This transpiration of water by plants is their main water use, with the amount of water used for photosynthesis very small by comparison. Thus, the main requirement for water is not directly for plant growth. Transpiration allows the transport of water and nutrients and the cooling of plant tissues (Kramer and Boyer, 1995; McKenzie *et al.*, 1999).

The rate of transpiration is correlated with the rate of photosynthesis. Plants open their stomata to absorb CO_2 for photosynthesis, but at the same time they also lose water through the stomata. When soil water is limiting plants can respond by closing their stomata to decrease water loss from transpiration, but this also decreases photosynthetic rate (McKenzie *et al.*, 1999). The water relations of white and Caucasian clovers are complex. The rate of transpiration is influenced by plant factors such as stomatal resistance, leaf water potential, and root morphology, and also by external factors such as atmospheric vapour pressure.

2.6.2.1 *Water status and plant growth*

A commonly accepted view with regard to the response of white clover to water stress is that the plant does not control leaf transpiration efficiently (Aparicio-Tejo *et al.*, 1980a, b; Burch and Johns, 1978; Johns, 1978). For example, in glasshouse conditions, Johns (1978) found that leaf conductance (G_l) was greater for white clover (~7.0 mm/s) than tall fescue (~4.0 mm/s) at a leaf water potential (Ψ_l) of –0.4 MPa (well-watered control), and declined in curvilinear relationships to a minimum of ~1.0 mm/s for both species under severe water stress conditions (Ψ_l = –5.1 MPa). The minimum G_l was mostly achieved by a Ψ_l of –3.0 MPa for tall fescue (1.5 mm/s), but leaf resistance ($1/G_l$) was not sufficient to prevent transpiration from occurring for white clover at the same Ψ_l (2.4 mm/s). This lack of leaf transpiration control in white clover was attributed to its inability to close stomata as well as tall fescue in response to short-term water stress.

The initial response of white clover to water stress is reduced elongation growth and fresh weight of leaves (Aparicio-Tejo *et al.*, 1980a, b; Blaikie *et al.*, 1988a). For example, under field conditions, relative (dryland/irrigated) petiole elongation rate (an indicator of leaf growth rate) in white clover decreased to 50% when Ψ_l (–1.1 MPa) was still similar in both dryland and irrigated plots, but decreased to 90% at a Ψ_l of –2.7 MPa under dryland conditions (Guobin and Kemp, 1992). This was caused by a decrease in cell growth or turgor. Cell growth is more sensitive to water stress than plant processes such as transpiration, photosynthesis, respiration, and N_2 fixation (Aparicio-Tejo *et al.*, 1980b; Johns, 1978; McKenzie *et al.*, 1999).

Leaf photosynthetic rate in white clover decreases with increasing water stress. Over a drying cycle after irrigation, canopy photosynthetic rate of white clover decreased linearly from 18.2 μmol $CO_2/m^2/s$ at a canopy conductance (G_c) of 12.0 mm/s to 9.1 μmol $CO_2/m^2/s$ at a G_c of 4.0mm/s (Blaikie *et al.*, 1988b). In the glasshouse study by Johns (1978), under uniform temperature (~20 °C) and light (~720 μmol/m^2/s PPFD) conditions, gross leaf photosynthetic rate in white clover was ~18.2 μmol $CO_2/m^2/s$ at a Ψ_l of –0.4 MPa and declined linearly to ~9.1 μmol $CO_2/m^2/s$ at a Ψ_l of –3.0 MPa. A similar response was found for tall fescue, which transpired less water, highlighting the inefficiency of water use by white clover.

Another response to short-term water stress in white clover is leaf senescence, which reduces leaf area and therefore transpiration loss. From the middle of a dry cycle, Ψ_l and leaf area index (LAI) values of white clover decreased. But until the end of the cycle the rate of transpiration loss per unit of leaf area was similar to that of tall fescue, which had more control of transpiration and higher Ψ_l, prolonging its LAI and growth (Burch and Johns, 1978). Leaf senescence in white clover was caused by its poor reduction in leaf conductance, and has been interpreted as a survival strategy for the protection of stolons under water stress conditions (Karsten and MacAdam, 2001; Turner, 1990a, b).

The inability of white clover to control transpiration can explain its low WUE under both dryland and irrigated conditions (Johns and Lazenby, 1973a, b). Leaf senescence as a means of preventing excessive transpiration was particularly evident and under summer conditions up to 70% of the herbage died in dryland pastures, and 20% of the herbage died in irrigated pastures (Johns and Lazenby, 1973a).

2.6.2.2 *Water status and plant development*

Relative to leaf and petiole elongation the rate of leaf appearance per stolon apex is less affected by short-term water stress. For example, under field conditions, relative (dryland/irrigated) leaf appearance rate per stolon apex of white clover decreased to 20% at a Ψ_l of –2.7 MPa under dryland conditions, compared with 90% for petiole extension at the same Ψ_l value and dryland conditions (Guobin and Kemp, 1992).

2.6.2.3 *Plant water extraction*

Root length and root density influence the ability of plants to access and extract the available soil water (Burch and Johns, 1978). Evans (1978) found that a mature white clover pasture had most of its roots in the top 0.20 m of soil, but could extract water to a depth of 0.90 m. In contrast, lucerne extracted water from at least 2.10 m. As a result, DM yield was greater for lucerne (15 120 kg/ha) than white clover (5420 kg/ha) over a 5-month period. Similar results have been reported for lucerne by Brown *et al.* (2003) in Canterbury New Zealand. Thus, the shallow nodal root system of white clover makes it susceptible to water stress, whereas a deep taproot system enables lucerne, and presumably Caucasian clover, to remain productive because of access to soil water at a greater depth.

2.6.2.4 Calculating water use of pasture species

The water use or evapo-transpiration (ET, evaporation from soil plus transpiration by plants) of pasture species can be either measured as actual evapo-transpiration (AET) by measuring changes in soil water content over time, or estimated by calculating the potential evapo-transpiration (PET). PET is regulated solely by atmospheric conditions when the following assumptions hold: the pasture is actively growing, short and green of uniform height, completely covering the ground over an extended area, adequately supplied with water and nutrients, and disease free (Penman, 1961). Under these circumstances PET and AET are usually the same. When soil water is limiting PET continues to increase, but AET deviates as water becomes more difficult to extract. PET can be calculated from measurements of certain meteorological parameters and recognition of simple physical principles (French and Legg, 1979; Johns and Lazenby, 1973a).

In NSW Australia, Johns and Lazenby (1973a) found that 1-year-old monocultures of white clover and three grass species used similar quantities of water (835 mm) in their second year under irrigated (non-limiting moisture) conditions. All four species also used similar quantities of water (564 mm) under dryland conditions (maximum soil moisture deficit of ~110 mm to 1.60 m depth) despite large differences in their ability to grow under such conditions (Section 2.6.2.5). For white clover, under irrigated conditions the rate of water use was 1.51 mm/d in July and increased to a maximum of 5.91 mm/d in December before it decreased to 2.01 mm/d in May. Water use by dryland white clover was 1.51 mm/d in July and increased to 3.53 mm/d in December before decreasing to 0.76 mm/d in May.

2.6.2.5 Calculating water use efficiency of pasture species

Whether water use is calculated or measured, the primary interest is the relationship between water use and DM production. This relationship represents the WUE or its reciprocal, the transpiration ratio. WUE can be defined using Equation 2.6.

Equation 2.6
$$WUE = \frac{DM}{ET}$$

WUE is affected by a number of factors, such as temperature, nutrient availability, and moisture availability, as well as the characteristics (i.e. stomatal control and leaf area) of individual pasture species. For example, Johns and Lazenby (1973b) reported that 1-year-old monocultures of white clover produced similar DM (12 840 kg/ha) to tall fescue (11 910 kg/ha) in their second year under irrigated conditions. However, tall fescue produced more DM (10 170 kg/ha) than white clover (6700 kg/ha) under dryland conditions. Thus, given the quantity of water used (Section 2.6.2.4), both species had similar WUE under irrigated conditions, but tall fescue had 25% greater WUE than white clover under dryland conditions. White clover WUE decreased by 35% under dryland conditions, indicating its high susceptibility to water stress as it is inefficient at limiting transpiration losses via stomatal closure. Also, WUE varied seasonally. White clover WUE averaged 1.2, 2.8, and 1.2 kg DM/mm of water used for periods November–December, February–March, and April–May, respectively, under irrigated conditions in NSW, Australia (Johns and Lazenby, 1973b).

The calculation of WUE has some limitations. One limitation is that ET includes soil evaporation water loss which does not contribute to DM production. The accuracy of determining WUE can be improved by using transpiration efficiency, which excludes evaporation (Tanner and Sinclair, 1983). Johns (1978) reported soil evaporation and pasture transpiration accounted for 3 and 97% of water used, respectively, by temperate pasture species. This 3% would equate to evaporation of 25 mm/yr if PET was 835 mm/yr.

Furthermore, water demand is affected by canopy leaf area and environmental factors such as solar radiation, temperature, and atmospheric humidity (the reciprocal which is the vapour pressure deficit (VPD)). When VPD increases, transpiration increases but there is no associated increase in the rate of photosynthesis. As a result, WUE decreases because more water is required to produce the same quantity of DM. However, under conditions of high atmospheric humidity (low VPD), WUE increases because the gradient between internal and external water vapour concentrations is less (Tanner and Sinclair, 1983). Thus, calculations of WUE can indicate the relative efficiency with which different pasture species are exploiting their water resources within an environment, but care must be taken when interpreting or extrapolating WUE from the literature.

2.7 Conclusions

1. Both Caucasian and white clovers differ in their growth and development characteristics. Specifically, white clover spreads via surface stolons which are dependent on shallow nodal roots after the seminal taproot has decayed. In contrast, Caucasian clover spreads via underground rhizomes, and taproots are deeper and persist longer than that of white clover. These morphological differences underpin the responses of each species to environmental and management factors.
2. Caucasian clover is more persistent than white clover under low temperature, water stress, and/or low soil fertility (pH, P and S availability) conditions, due to its extensive root and rhizome system. However, both species respond to P, S and lime. Additional research is required on the seasonal productivity of Caucasian clover on low fertility and dryland soils under intensive lowland conditions.
3. Caucasian clover is very persistent under grazing, has a high N_2 fixation ability, and a high forage nutritive value which is similar to that of white clover. Thus, any increase in total clover production by the inclusion in pastures of Caucasian clover is likely to increase animal performance and N yield.
4. Plant growth and development responses to temperature and water supply provide explanations for the seasonal productivity of white clover under irrigated and dryland conditions. However, there is no information on the physiological basis for the agronomic responses of Caucasian clover under these conditions.
5. Caucasian clover is slower to establish than white clover. This has been attributed to its priority towards root and rhizome development, which makes it sensitive to competition from other species during establishment. Further research is required to devise the most efficient establishment method for Caucasian clover under lowland conditions.

Based on these conclusions the four primary objectives outlined in Section 1.2 and Figure 1.1 were developed.

Chapter 3

Sheep liveweight gain on Caucasian clover–ryegrass and white clover–ryegrass pastures under high and low soil fertility conditions

3.1 Introduction

In Chapter 2, the literature review identified soil fertility as a key factor in the adaptation of legumes. The most important mineral elements required for legumes in temperate pastures are P and S (During, 1984). In long-term (>5 years) experiments Caucasian clover has been shown to persist longer than white clover under low soil fertility conditions. For example, on a soil with an Olsen P of 8 μg/ml and pH of 5.2 in New Zealand (South Island) high country, 9-year-old Caucasian clover pastures were more productive than white clover pastures (Daly and Mason, 1987). A feature of Caucasian clover's persistence in tussock grasslands is its ability to survive with only occasional (i.e. every 4 years) applications of P, S and Mo fertiliser (Strachan *et al.*, 1994; Virgona and Dear, 1996; White, 1995). However, the ability to persist and out-perform white clover under low soil fertility conditions in more productive lowland perennial ryegrass pastures has not been reported. If Caucasian clover has an advantage over white clover in these soil conditions then Caucasian clover pastures can be expected to have a greater legume content. Assuming Caucasian clover has a similar nutritive value to white clover (Allinson *et al.*, 1985) then any increase in clover content should also increase animal performance (Askin *et al.*, 1987; Hyslop *et al.*, 2000; Stevens *et al.*, 1993).

Thus, the objective of this research was to use sheep performance on Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures to assess the relative legume performance under high (High-F) and low (Low-F) soil fertility conditions. Results presented include sheep liveweight gain (LWG) per hectare, average daily liveweight gain (ADLWG) and number of grazing days. Pasture mass, botanical composition and nutritive value measurements were used to explain differences between treatment flocks.

3.2 Materials and methods

3.2.1 Site description

3.2.1.1 Location

The experiment area was located in block H17 of the Field Service Centre research area at Lincoln University, Canterbury, New Zealand (43° 39'S, 172° 28'E, 11 m a.s.l.). The experimental area is flat with small (~1 m) changes in topography. Tall (8–10 m) poplar (*Populus deltoides* x *nigra*) shelterbelt trees are present on the west and north boundaries.

3.2.1.2 Soil

The soil is a Templeton silt loam (Udic Haplosteps, USDA Soil Taxonomy) with 1–2 m of fine textured alluvial sediments overlying gravels (Cox, 1978). The typical profile texture consists of 0.3 m of uniform topsoil underlain by layers of varying depth ranging from silt loam to sand in texture. Depths to gravels at the site have been shown to range from 0.6 to 1.5 m (Gyamtsho, 1990). Templeton silt loam soils are medium to free-draining with a moderate water-holding capacity of ~300 mm in the top 1.0 m (Cox, 1978; Watt and Burgham, 1992).

3.2.1.3 Meteorological conditions

3.2.1.3.1 <u>Rainfall, evapo-transpiration and irrigation</u>

This experiment was conducted over 3 years (Years 2, 3, and 4 of the experiment). Year 2 (1998/99) and Year 4 (2000/01) were the driest with annual rainfall (~480 mm from 1 July to 30 June) ~28% below the long-term mean (LTM) of 670 mm, but PET was similar to the LTM of 1060 mm (Table 3.1). In Year 3 (1999/00) annual rainfall was similar to the LTM and PET was 11% below the LTM. The amount of irrigation water applied ranged from 400 mm in 1998/99 to 300 mm in 2000/01. Details of irrigation management are given in Section 3.2.7. The potential soil water deficit (PSWD) varied from 150 mm in 1999/00 to 288 mm in 2000/01.

Table 3.1 Rainfall, Penman potential evapo-transpiration (PET), potential soil water deficit (PSWD) and the amount and timing of irrigation water applied for three years (1 July–30 June) and the long-term mean (LTM) for block H17 at Lincoln University, Canterbury, New Zealand.

Year	Rainfall (mm)	PET (mm)	PSWD (mm)	Irrigation (mm)	Timing of irrigation
1998/99	475	1057	215	400	16 Nov.–15 Mar.
1999/00	687	946	150	300	22 Nov.–10 Mar.
2000/01	485	1048	288	350	14 Nov.–31 Mar.
LTM	670	1060	478		

Note: Rainfall and PET data were obtained from Broadfields meteorological station located 3 km north of the site.

Monthly rainfall was variable over the three seasons, ranging from 135 mm in July 1999 to ~6 mm/month in February–April 2001 (Figure 3.1). Monthly PET followed a similar pattern in each season, increasing from a minimum of ~30 mm in July to a maximum of between 130 and 180 mm in December before decreasing to a minimum again in June. The PSWD generally began to increase in September of each season (Figure 3.2), but the timing and extent of PSWD was dependent on rainfall and irrigation. In 2000/01 the PSWD exceeded 200 mm in February.

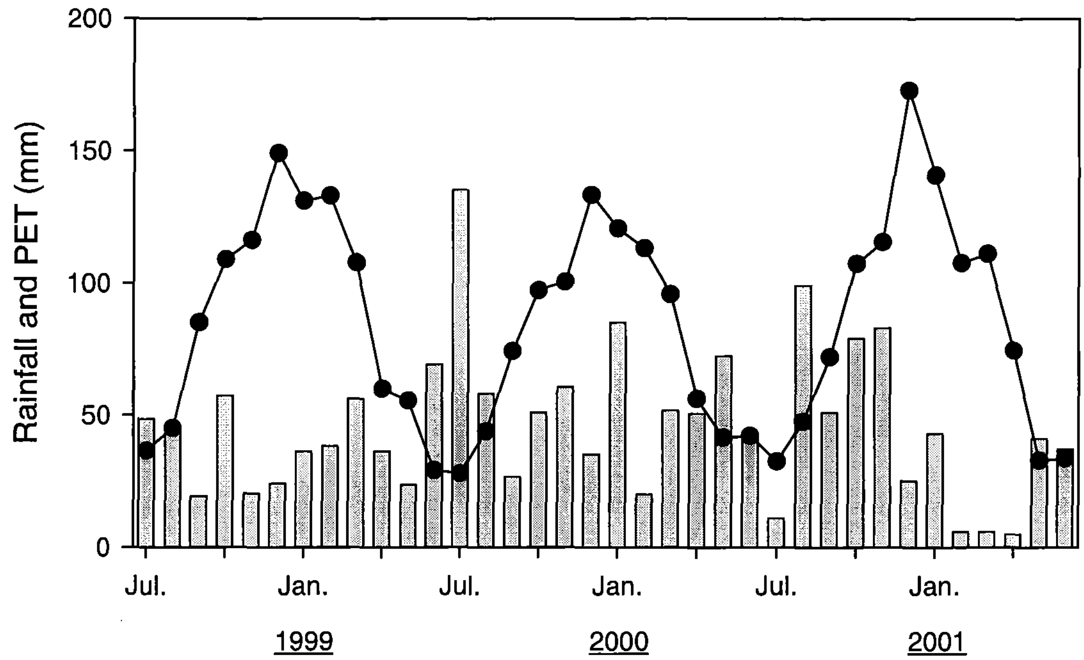

Figure 3.1 Monthly rainfall (bars) and Penman potential evapo-transpiration (PET, •) from 1 July 1998 to 30 June 2001. Data were obtained from Broadfields meteorological station (3 km north of the site), Canterbury, New Zealand.

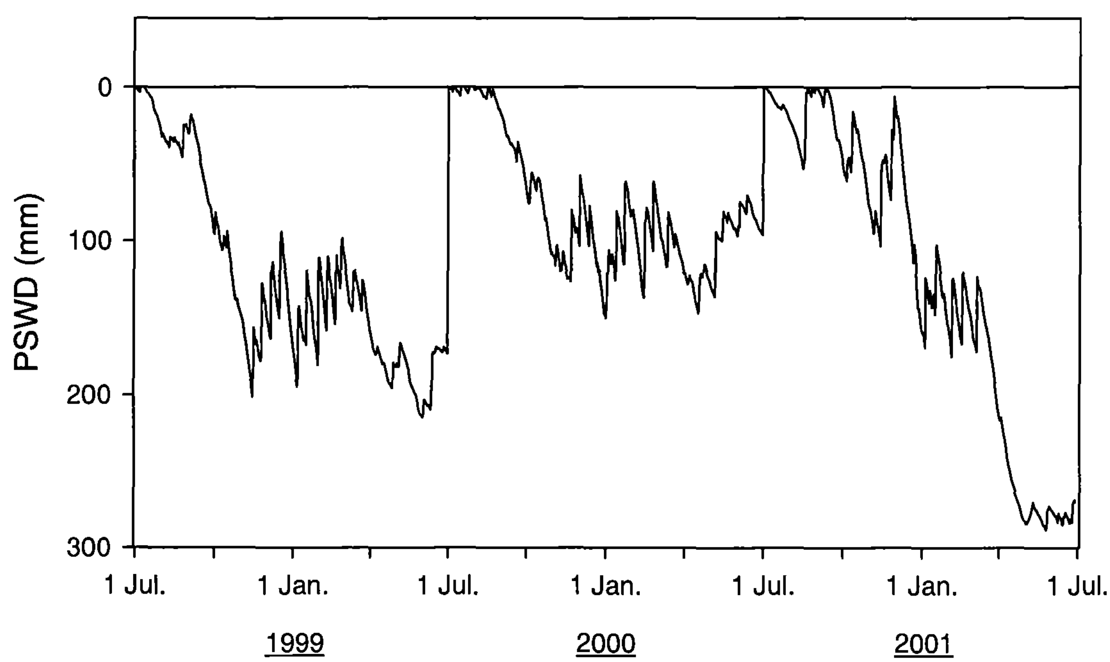

Figure 3.2 Daily potential soil water deficit (PSWD) calculated from 1 July 1998 to 30 June 2001 for block H17 at Lincoln University. Rainfall and Penman evapo-transpiration data were obtained from Broadfields meteorological station (3 km north of the site), Canterbury, New Zealand.

3.2.1.3.2 Temperature and solar radiation

The mean daily air temperature followed a similar pattern in each season, ranging from 6–8 °C in June–August to 15–17 °C in February (Figure 3.3). The mean daily 10 cm soil temperature ranged from 5–7 °C in June–August to 18–21 °C in January/February. The mean daily total solar radiation followed a similar pattern each season, increasing from a minimum of 4–6 $MJ/m^2/d$ in June/July to a maximum of ~25 $MJ/m^2/d$ in December.

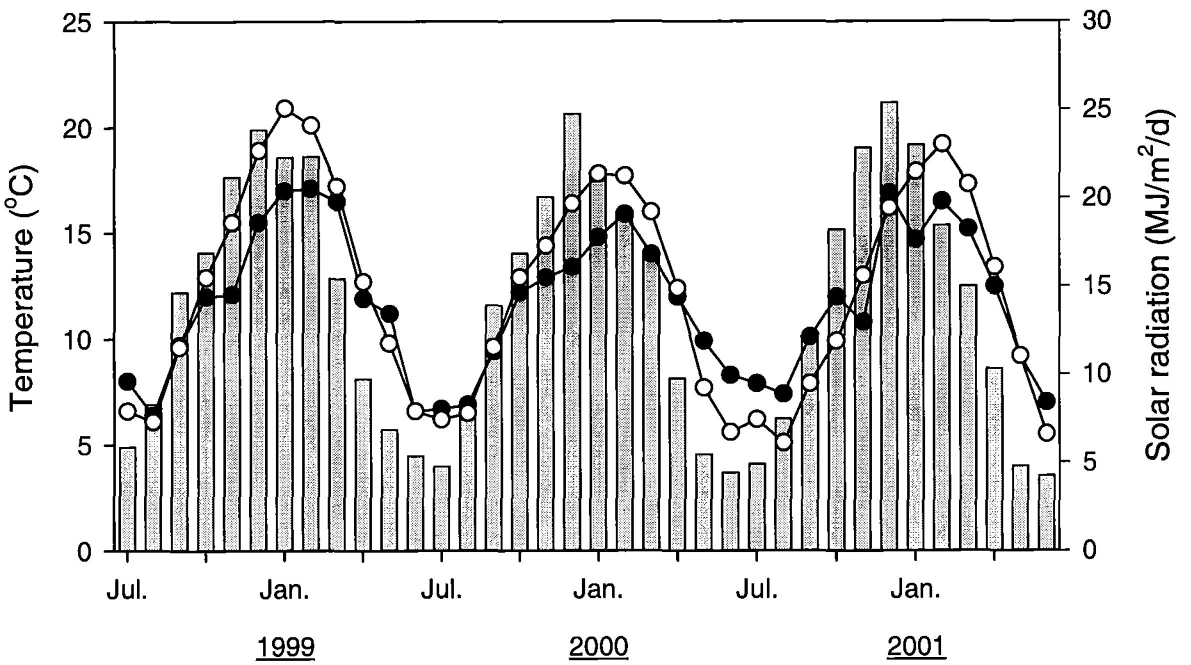

Figure 3.3 Mean daily air (•) and 10 cm soil (○) temperatures, and mean daily solar radiation (bars) from 1 July 1998 to 30 June 2001. Data were obtained from Broadfields meteorological station (3 km north of the site), Canterbury, New Zealand.

3.2.2 Pasture establishment

The experiment used a 2^2 factorial in a Latin square design. Treatments were CC–RG and WC–RG pastures, and High-F and Low-F soil fertility conditions. There were eight replicates of pasture treatments (32 plots) and two replicates of sheep flocks (eight flocks). Plots were 29 x 14 m or 0.04 ha.

The experimental area had previously been used for a 7-year experiment on lucerne/grass mixtures (Gyamtsho, 1990; McKenzie *et al.*, 1990) but had been out of pasture for ~2 years prior to this experiment. Prior to sowing, the experimental area was cultivated using conventional methods to produce a firm and fine textured seedbed. The pre-plant soil incorporated herbicide 'Triflur 40' (a.i. trifluralin at 1.0 kg a.i./ha) was sprayed the day before sowing to control weeds.

On 11 December 1996, 'Grasslands Demand' white clover (2 kg/ha) and the hexaploid Caucasian clover cultivar 'Endura' (6 kg/ha) were sown as pure species. Caucasian clover seed was inoculated with the *Rhizobium* strain ICC148. Plots were drilled with an Øyjoord cone seeder at 150 mm row spacing and a target depth of 15 mm. On 27 January 1997, the post-emergence herbicide 'Basagran' (a.i. bentazone at 1.4 kg a.i./ha) was sprayed to control mainly shepherd's purse (*Casella bursa-pastoris*) and fathen (*Chenopodium album*).

Both clovers established well with 2800–3000 kg DM/ha accumulated after 4 months when plots were first grazed. On 10 March 1997, after a hard grazing, 'Grasslands Ruanui' zero endophyte perennial ryegrass (15 kg/ha) was direct-drilled into the clover monocultures. Both clovers made significant contributions to botanical composition by October 1997.

3.2.3 Soil fertility

Before the experiment was established, the soil had a pH of 5.9, an Olsen P of 11 μg/ml and a sulphate-S of 6 μg/g (Table 3.2). In December 1996, all plots received lime at 1 t/ha with Mo.

In December 1996 and August 1997, superphosphate (8% P, 12% S) fertiliser at 600 kg/ha was applied to the High-F treatment. Soil fertility was monitored by soil tests taken in May of each season from 1998 to 2001. Soil tests involved 15 soil cores (0–75 mm) taken from each plot and bulked into two replicates to represent soil from the farmlets grazed by each of the eight treatment flocks. Sub-samples were analysed using Ministry of Agriculture and Fisheries Quick Test (MAF QT) procedures. Data from each season are given in Appendix 1 and 4-year means are presented in Table 3.2.

Table 3.2 Soil test (0–75 mm) results for block H17 from 1996–2001 at Lincoln University, Canterbury, New Zealand. August 1996 results are from the site before establishment. May 1998–2001 results are 4-year means from Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions.

		pH (H_2O)	Olsen P (μg/ml)	SO_4-S (μg/g)	Ca^{++}	K^+ (meq/100 g)	Mg^{++}	Na^+
August 1996								
		5.9	11.0	6.0	–	0.7	–	–
May 1998–2001								
High-F	CC–RG	6.0	20.8_a	11.4_b	7.3	0.87_{ab}	1.04	0.23
	WC–RG	6.0	18.5_a	13.3_a	7.5	0.77_b	1.02	0.26
Low-F	CC–RG	6.1	10.9_b	7.0_c	7.3	0.90_a	1.09	0.25
	WC–RG	6.1	10.4_b	7.1_c	7.4	0.88_{ab}	1.08	0.26
s.e.m.		0.04	0.40	0.07	0.11	0.026	0.024	0.010
P_C		0.326	0.040	<0.001	0.211	0.098	0.533	0.132
P_F		0.065	<0.001	<0.001	0.656	0.073	0.108	0.435
$P_{C \times F}$		0.878	0.114	0.001	0.590	0.267	0.793	0.332

Note: Soil samples were analysed using Ministry of Agriculture and Fisheries Quick Test (MAF QT) procedures. Subscript C = clover species and F = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different ($\alpha = 0.05$).

The aim of the High-F treatment was to maintain the soil at an Olsen P level of ~20 μg/ml and at a sulphate-S level of ~10 μg/g, respectively, according to published relationships between relative pasture production and Olsen P or sulphate-S levels for sedimentary soils (Morton and Roberts, 1999). This required two applications of sulphur superphosphate (8% P, 19% S) fertiliser at 250 kg/ha to the High-F treatment in November 1999 and November 2000. Average soil test results in May indicated that target levels of Olsen P and sulphate-S had been achieved for the High-F treatment (Table 3.2). No fertiliser was applied to the Low-F treatment which had Olsen P levels that ranged between 9 and 13 μg/ml and sulphate-S levels that ranged between 5 and 9 μg/g from the start of the experiment in 1996 to May 2001 (Appendix 1).

3.2.4 Animals

The experiment used weaned 'Coopworth' ewe lambs selected on animal soundness and initial live weight in February each year. The shorn lambs were stratified into 10 uniform weight groups with eight lambs in each. From each of these weight groups, lambs were assigned randomly to the eight treatment flocks. Thus, each flock consisted of one lamb from each weight group to give 10 lambs per treatment flock, ear-tagged according to their respective treatment flock and initial weight group. Sheep were shorn as hoggets in summer and replaced by new lambs in autumn. Sheep were treated as necessary with an oral anthelmintic drench to control internal parasites and pour-on dip for lice control.

3.2.5 Grazing management

In Year 1 of this experiment (1997/98) pastures were grazed as necessary with mixed age sheep in spring/summer. From then on the pastures were grazed with the eight treatment flocks. Each flock was managed on a four plot rotation within 0.16 ha farmlets of the same treatment. Rotations were relatively short (14–24 d) in spring and longer (19–31 d) in summer/autumn to provide grazing management suitable for both clovers. Details of grazing rotations are given in Appendix 2.

A variable stocking method (Bransby and Maclaurin, 2000) was used to maintain all four treatments at a similar pasture allowance within a range of 1.5 to 2.0 kg DM/head/d at entry into a new plot in each treatment. Flock size adjustments were made at 19–39 d intervals at each weighing, and all adjustments required removal but not addition of sheep. Flock size (± standard error) averaged 7.5 ± 0.49 hoggets in spring/summer and 6.3 ± 0.78 lambs in autumn. Each flock had a minimum of four measurement sheep.

Flock size adjustments maintained a measured mean pre-grazing pasture mass of 2000–2600 kg DM/ha and a measured mean post-grazing pasture mass of 900–1500 kg DM/ha in each farmlet. If the mean pre-grazing pasture mass was less than 2000 kg DM/ha for a farmlet then all farmlets were destocked for 7–14 d and sheep LWG measurements were recommenced when the mean pasture mass recovered. After the final rotation in May all lambs were massed to graze outside the experimental area in winter before returning to their treatment flocks in September as hoggets.

Small flocks (4–6 sheep) of spare sheep were placed in perimeter and internal raceways to reduce the effects of camp behaviour and nutrient transfer by treatment flocks in the plots. The sequence by which treatment flocks grazed plots was altered each year to reduce the previous pattern of camp behaviour which was most obvious in the perimeter plots.

3.2.6 Grazing periods

Data from this experiment are summarised into spring and autumn grazing periods (Table 3.3). The spring grazing period began when a mean pre-grazing pasture mass of 2000–2600 kg DM/ha had accumulated in all treatments. The end of each spring grazing period coincided with shearing of the hoggets in December/January. The autumn period began when lambs were introduced to the experiment in February each year, and finished when pasture growth rates were unable to meet the required pre-grazing pasture mass.

Sheep grazed pastures after shearing in summer, but sheep LWG were not measured because the near mature hoggets had a mean (± standard error) individual live weight of 61.5 ± 0.37 kg which would have had limited their LWG potential. Thus, animals in summer were used primarily to control reproductive stem development of ryegrass and invasive grasses.

Table 3.3 Grazing period start and finish dates and duration from 11 September 1998 to 1 May 2001 in block H17 at Lincoln University, Canterbury, New Zealand.

Year	Spring			Autumn		
	Start date	Finish date	Duration (d)	Start date	Finish date	Duration (d)
1998/99	11 Sep.	18 Dec.	98	5 Feb.	10 Jun.	111
1999/00	14 Sep.	5 Jan.	90	11 Feb.	23 May	86
2000/01	14 Sep.	13 Dec.	89	12 Feb.	1 May	78

Note: Liveweight gain was not measured from 11 April 2001 to 1 May 2001 as described in Section 3.2.10.2.

3.2.7 Irrigation

Plots were spray irrigated with the aim of applying about 100 mm of irrigation water plus rainfall per month from November to March. In 1997/98 (Year 1) plots received about 400 mm of irrigation water. The amount and timing of irrigation water applied for the following three seasons are given in Table 3.1 (Section 3.2.1.3.1).

3.2.8 Insect pests

Insect pests caused severe damage to all pastures over the three growing seasons from September 1998 to June 2001. The lack of endophytic fungi in 'Grassland Ruanui' perennial ryegrass meant that it probably suffered damage by Argentine stem weevil (*Listronotus bonariensis*). However, the parasitiod *Microtonus hyperodae*, introduced to the area in 1991, may have reduced the incidence of attack by Argentine stem weevil (McNeill *et al.*, 2002).

Grass grub (*Costelytra zealandica*) caused noticeable damage to all pastures in autumn 1999. Visual assessment of grass grub damage on 26 March 1999 showed that about 32–44% of the experimental area was damaged by grass grub (Amyes, 1999). Grass grub populations were about 180/m^2 for all pasture treatments. The insecticide 'Diazinon' (a.i. diazinon at 3.0 kg a.i./ha) was applied to all plots in April 1999 to control the grass grub. Also, ryegrass was severely damaged by pasture mealy bug (*Balanococcus poae*) in autumn 2001 but this could not be treated.

3.2.9 Mowing

Pastures were mown or 'topped' post grazing as necessary in late spring/summer in 1999/00 and 2000/01 to reduce ryegrass and invasive grasses reproductive stem development. Invasive grasses comprised mostly barley grass (*Hordeum* ssp.) and smooth brome grass (*Bromus hordeaceus* syn *B. mollis*).

3.2.10 Measurements

3.2.10.1 Meteorological conditions

Meteorological data (Section 3.2.1.3) were obtained from the Broadfields meteorological station (Crop & Food Research Ltd.) located 3 km north of the site. The potential soil water deficit (PSWD) was calculated throughout each season using the equation presented by French and Legg (1979), but with irrigation included:

Equation 3.1 $$PSWD = PSWD_{i-1} + PET - (R + I)$$

where $PSWD_{i-1}$ is the PSWD on the previous day. PSWD was set to zero at the start of each season (1 July) and was not allowed to exceed zero (i.e. field capacity). PET is Penman potential evapo-transpiration, and R and I are rainfall and irrigation, respectively.

3.2.10.2 Sheep liveweight gain (LWG) per hectare, average daily liveweight gain (ADLWG), and number of grazing days

All sheep were weighed at 19–32 d intervals, usually at the end of each rotation in autumn and every second rotation in spring. Fasted live weights were measured immediately prior to and at the completion of each spring and autumn grazing period (Section 3.2.6). Unfasted live weights were measured between fasted live weight measurements. ADLWG was calculated using data from the measurement animals, and LWG per hectare was obtained from the liveweight changes of all animals. LWG was not measured from 11 April to 1 May 2001 when pastures were deliberately grazed to a low post-grazing mass of 800–1200 kg DM/ha with a pasture allowance of 1.0–1.5 kg DM/head/d. The number of grazing days per hectare (i.e. number of sheep/ha × number of days grazed) was recorded for each grazing period.

3.2.10.3 Pre-grazing pasture mass

Pre-grazing pasture mass was measured the day animals were introduced to each plot. Post-grazing pasture mass was measured the day animals were removed, and is described in Section 3.2.5. Measurements were made using a capacitance probe (Mosaic Systems Ltd., New Zealand). To minimise the coefficient of variation for pasture mass, each plot was divided into five equal areas or 'strata' and 10 probe readings were taken in each 'strata' (Cayley and Bird, 1996).

The probe was calibrated on five occasions throughout each year to account for changes in botanical composition. Separate calibration equations were established for each treatment and pre- and post-grazing pasture mass. For each calibration, paired samples of probe reading and measured herbage mass were taken from 0.2 m^2 quadrat cuts to 30 ± 2 mm above ground across a range of herbage mass. Samples were dried at 70 °C for at least 24 h and weighed. The paired samples were then used to establish a linear equation by regression between capacitance probe readings and herbage mass.

3.2.10.4 Botanical composition

Botanical composition was measured on the day animals were introduced to each plot, by taking 25 'snip' samples (i.e. 50 g fresh weight samples cut to 30 mm above ground) of herbage across each plot using hand shears. Five samples were taken from each of the five 'strata' in each plot and mixed together before sub-sampling. The fresh sub-samples, containing 200–400 pieces, were dissected into white clover, Caucasian clover, perennial ryegrass, invasive grasses, broadleaf weeds and dead material before dry weight of each component was measured. Some volunteer white clover (presumably 'Grasslands Huia') was detected in the Caucasian clover treatments in 1999/00 and 2000/01. The invasive grasses were mostly barley grassand smooth brome grass.

3.2.10.5 Nutritive value

The dried sub-samples of each clover species and grass were retained for determination of nutritive value. Ryegrass and clover samples from each plot were bulked into two replicates to represent herbage from the farmlets grazed by each of the eight treatment flocks. Bulked samples were ground in a mill to pass through a 1-mm stainless steel sieve (Cyclotec Mill, USA). The crude protein (CP) concentration was obtained as N concentration × 6.25 using the Kjeldahl procedure (Kjltec Auto 1030 Analyser, Tectator, Sweden). The metabolisable energy (ME) concentration was obtained from the concentration of digestible organic matter in the dry matter (DOMD) × 0.16. The DOMD concentration was determined using an *in vitro* cellulase-based method (Adesogan *et al.*, 2000). Analyses were performed by the Animal and Food Sciences Division, Lincoln University.

3.2.10.6 Herbage mineral concentrations

Samples of clover leaf plus petiole were hand-plucked in December 1998 and 1999, and as well as ryegrass leaf plus pseudostem in November 2001, for determination of herbage mineral (N, P, S, Mg, Ca, Na, K) concentrations. Samples were collected at ~20 d regrowth, bulked into two replicates according to the eight treatment flocks, dried and ground. Analyses were performed by Celentis Analytical (AgResearch Ltd., New Zealand). In addition, clover leaf plus petiole samples collected in December 1998 were analysed for micronutrients and these results are given in Appendix 6.

3.2.11 Statistical analysis

Data were analysed using analysis of variance (ANOVA) procedures (GenStat, 1997). Years were treated as repeated measurements (Gomez and Gomez, 1984). The variability of annual and seasonal data was expressed as the standard error of the mean (s.e.m.) for the 3 years of measurement. Treatment means were compared using Fisher's protected least significant difference (l.s.d.) test whenever the ANOVA indicated that differences among treatments presented $P<0.05$ for all variables, except individual sheep LWG and nutritive value variables which used $P<0.10$. To satisfy the requirement for constant variance in ANOVA, botanical composition percentages were arcsine transformed when values were outside a range of either 0–30% or 70–100% (Sokal and Rohlf, 1981). Data are presented back-transformed with 95% confidence intervals calculated on the transformed data because the non-linear transformation meant that back-transforming the s.e.m. was not a valid option.

3.3 Results

3.3.1 Sheep liveweight gain (LWG) per hectare

Mean 1998–2001 annual sheep LWG per hectare was 10% greater (*P*<0.001) for CC–RG than WC–RG pastures under High-F conditions (Table 3.4). Sheep LWG per hectare was 7% lower (*P*<0.001) for CC–RG pastures under Low-F conditions, but the advantage (*P*<0.001) over WC–RG pastures was similar at 8%. In 1998/99, there were no significant differences in LWG per hectare between all four treatments. In 1999/00, sheep LWG per hectare was 16% greater (*P*<0.001) for CC–RG than WC–RG pastures, and 9% greater (*P*<0.001) for High-F than Low-F conditions. In 2000/01, the advantage was 8% (*P*<0.001) for CC–RG pastures, and 7% (*P*<0.01) for High-F treatments.

Table 3.4 Sheep liveweight gain per hectare from Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		kg/ha			
High-F	CC–RG	1243	1286_a	1005_a	1178_a
	WC–RG	1198	1101_c	909_b	1069_b
Low-F	CC–RG	1190	1173_b	919_b	1094_b
	WC–RG	1162	1016_d	868_b	1015_c
s.e.m.		21.5	9.1	19.0	12.7
P_C		0.107	<0.001	0.001	<0.001
P_F		0.055	<0.001	0.004	<0.001
$P_{C \times F}$		0.710	0.145	0.251	0.256

Note: Details of soil fertility treatments are given in Section 3.2.3. Subscript *C* = clover species and *F* = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different ($\alpha = 0.05$).

Treatment differences in sheep LWG per hectare occurred during both spring and autumn, except in spring 1998 when there were no significant differences between all four treatments (Figure 3.4). In spring 1999, sheep LWG per hectare was 16% greater (*P*<0.001) for CC–RG than WC–RG pastures, and 6% greater (*P*<0.001) for High-F than Low-F pastures. In spring 2000, the advantage was ~6% (*P*<0.05) for CC–RG pastures, and 5% (*P*<0.10) for High-F pastures. In autumn 1999, sheep LWG per hectare was 15% greater ($P<0.001$) from High-F than Low-F pastures. In autumn 2000, the advantage was 17% ($P<0.001$) for CC–RG pastures, and 14% ($P<0.001$) for High-F pastures. In autumn 2001, sheep LWG per hectare was 21% greater ($P<0.05$) for CC–RG than WC–RG pastures under High-F conditions, but this advantage was lower under Low-F conditions at 9%. Sheep LWG per hectare in autumn 1999 and autumn 2000 was ~50% greater than in autumn 2001 for all treatments, because animal performance was not measured from 11 April to 1 May 2001 (Section 3.2.10.2).

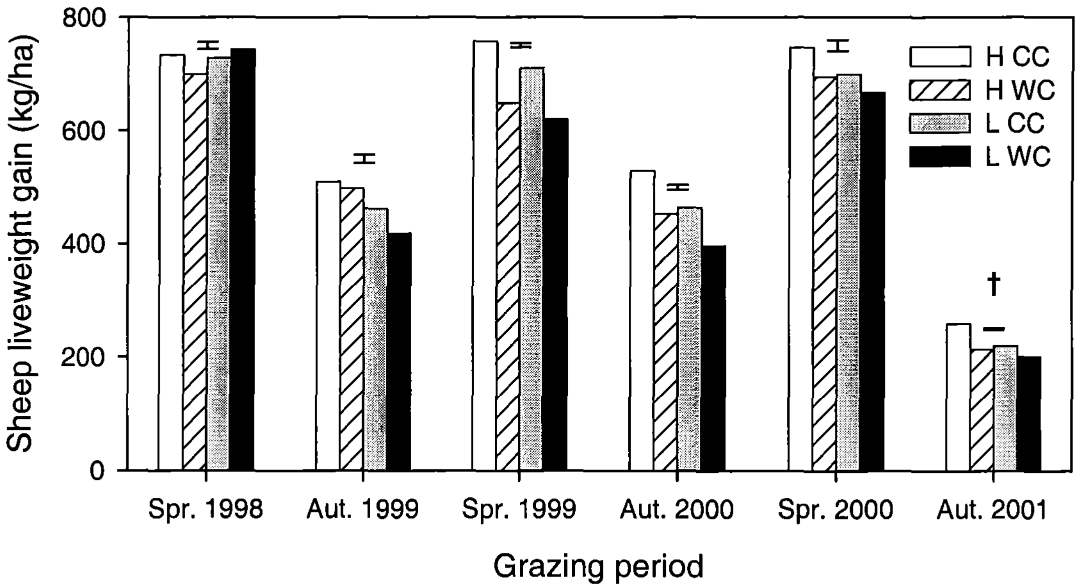

Figure 3.4 Sheep liveweight gain per hectare from Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions per grazing period from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean. †Liveweight gain was not measured from 11 April to 1 May 2001.

3.3.2 Average daily liveweight gain (ADLWG)

Mean 1998–2001 sheep ADLWG was 141 g/head/d on CC–RG pastures compared with 129 g/head/d ($P<0.05$) on WC–RG pastures (9% higher), but this was unaffected by soil fertility (Table 3.5). In 1998/99, there were no significant treatment differences. In 1999/00, ADLWG was 15% higher ($P<0.001$) for CC–RG than WC–RG pastures. In 2000/01, the advantage was 8% ($P<0.01$) for CC–RG pastures, and High-F treatments supported a 4% ($P<0.05$) higher ADLWG than Low-F treatments.

Table 3.5 Average daily liveweight gain of sheep on Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		g/head/d			
High-F	CC–RG	148	148_a	133_a	143_a
	WC–RG	143	127_b	121_c	130_b
Low-F	CC–RG	143	146_a	126_b	138_a
	WC–RG	139	128_b	119_c	128_b
s.e.m.		5.4	1.1	1.0	2.3
P_C		0.453	<0.001	0.002	0.015
P_F		0.476	0.764	0.018	0.257
$P_{C\times F}$		0.949	0.398	0.090	0.598

Note: Details of soil fertility treatments are given in Section 3.2.3. Subscript C = clover species and F = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different ($\alpha = 0.10$).

In spring 1998, there were no significant treatment differences in ADLWG, but in spring 1999 ADLWG was 16% higher ($P<0.01$) for CC–RG than WC–RG pastures (Figure 3.5). In spring 2000, the ADLWG advantage was 5% ($P<0.01$) for CC–RG pastures, and High-F conditions supported a 3% higher ($P<0.10$) ADLWG than Low-F conditions. In autumn 1999, ADLWG was 13% higher ($P<0.05$) under High-F than Low-F conditions, and in autumn 2000 it was 16% higher ($P<0.05$) for CC–RG than WC–RG pastures. In autumn 2001, the ADLWG advantage for CC–RG pastures was 14% ($P<0.05$). The ADLWG in autumn 2001 was ~40% lower than in autumn 1999 for all treatments.

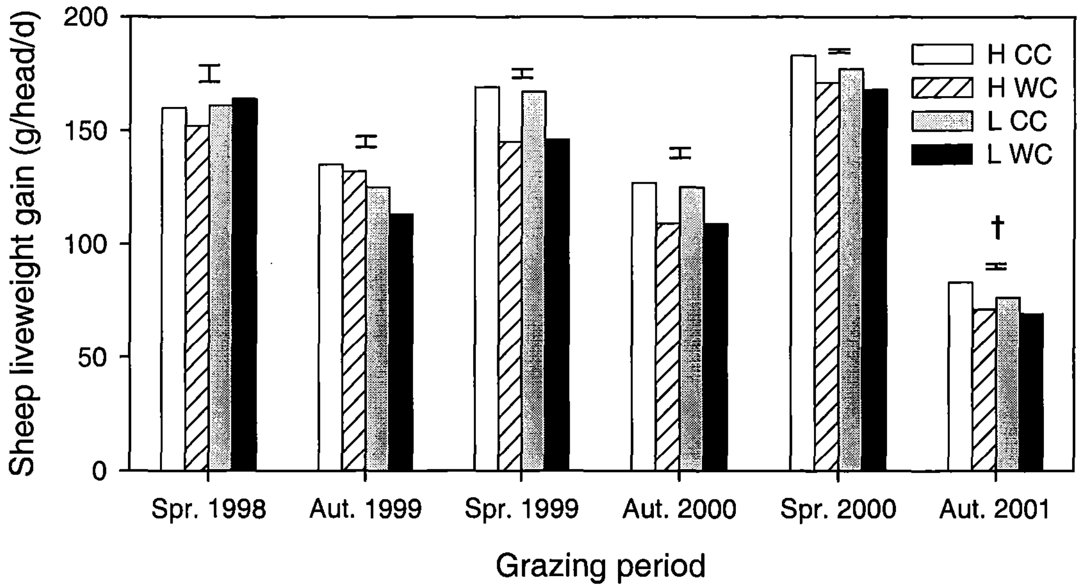

Figure 3.5 Mean individual sheep liveweight gain on Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions per grazing period from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean. †Liveweight gain was not measured from 11 April to 1 May 2001.

3.3.3 Number of grazing days

The mean 1998–2001 number of sheep grazing days was 5% greater (P<0.001) on High-F than Low-F soils, but was unaffected (P<0.644) by clover species (Table 3.6). In 1998/99, the number of grazing days averaged 8303 /ha for all four treatments. In 1999/00, the High-F treatments had 9% more (P<0.001) grazing days than the Low-F treatments, and in 2000/01 the advantage was 4% (P<0.05) for High-F treatments.

Table 3.6 Mean number of sheep grazing days for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		n/ha			
High-F	CC–RG	8362	8650_a	7175	8062_a
	WC–RG	8359	8628_a	7085	8024_a
Low-F	CC–RG	8236	7941_b	6869	7682_b
	WC–RG	8254	7876_b	6869	7666_b
s.e.m.		105.9	60.5	108.6	57.4
P_C		0.942	0.481	0.682	0.644
P_F		0.293	<0.001	0.030	<0.001
$P_{C\times F}$		0.925	0.724	0.682	0.848

Note: Details of soil fertility treatments are given in Section 3.2.3. Subscript C = clover species and F = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different (α = 0.05).

The number of sheep grazing days under High-F conditions was 6% more (P<0.001) than that under Low-F conditions in spring 1999, but there were no significant treatment differences observed in spring 1998 and spring 2000 (Figure 3.6). In autumn 1999, there were no significant treatment differences, but in autumn 2000 the number of grazing days under High-F conditions was 13% more (P<0.001) than under Low-F conditions. In autumn 2001, there was an interaction (P<0.05) between clover and soil fertility treatments; the number of grazing days was 3% greater for CC–RG than WC–RG pastures under High-F conditions, but was similar for both clovers under Low-F conditions. The number of sheep grazing days in autumn 1999 and autumn 2000 was ~28% greater than in autumn 2001.

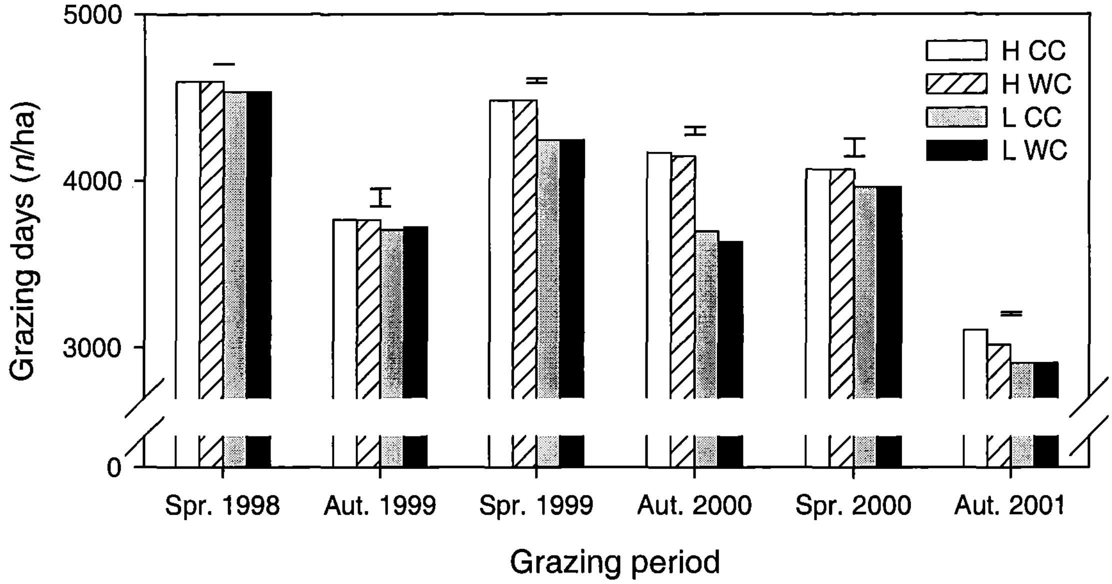

Figure 3.6 Number of sheep grazing days for Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions per grazing period from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the means.

3.3.4 Pre-grazing pasture mass

Mean 1998–2001 pre-grazing pasture mass was 5% greater (*P*<0.01) under High-F than Low-F conditions, but was unaffected (*P*<0.362) by clover species (Table 3.7). In 1998/99, pre-grazing pasture mass averaged 2230 kg DM/ha for all four treatments, but in 1999/00 it was 7% greater (*P*<0.01) under High-F than Low-F conditions. In 2000/01, the advantage was 4% (*P*<0.05) for High-F conditions.

Table 3.7 Mean pre-grazing pasture mass of Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		kg DM/ha			
High-F	CC–RG	2250	2590_a	2190_a	2340_a
	WC–RG	2270	2520_a	2100_{ab}	2300_{ab}
Low-F	CC–RG	2190	2390_b	2070_b	2220_b
	WC–RG	2210	2370_b	2060_b	2210_b
s.e.m.		36	45	31	30
P_C		0.733	0.287	0.154	0.362
P_F		0.119	0.002	0.028	0.004
$P_{C\times F}$		0.970	0.653	0.245	0.515

Note: Details of soil fertility treatments are given in Section 3.2.3. Subscript *C* = clover species and *F* = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different (α = 0.05).

Mean pre-grazing pasture mass was 5% greater (*P*<0.05) under High-F than Low-F conditions in spring 1999, but no significant treatment differences were observed in spring 1998 and spring 2000 (Figure 3.7). In autumn 1999, there were no significant treatment differences, but in autumn 2000 pre-grazing pasture mass was 12% greater (*P*<0.001) under High-F than Low-F conditions. In autumn 2001, pre-grazing pasture mass was 7% greater (*P*<0.01) for CC–RG than WC–RG pastures, and 5% greater (*P*<0.05) under High-F than Low-F conditions.

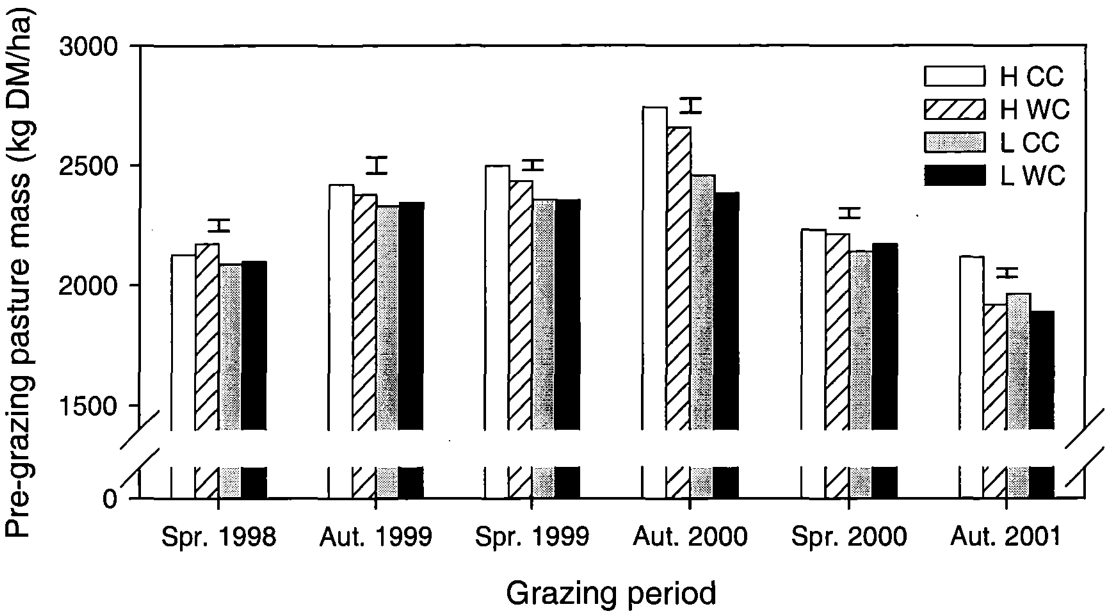

Figure 3.7 Pre-grazing pasture mass of Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions per grazing period from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

3.3.5 Botanical composition

The mean 1998–2001 total clover content (sown clover plus volunteer white clover) on offer in CC–RG pastures (20%) was approximately double ($P<0.001$) that for WC–RG pastures (11%), and was unaffected ($P<0.47$) by soil fertility (Figure 3.8). Volunteer white clover (presumably 'Grasslands Huia') detected in CC–RG pastures averaged 4% of the total DM. Ryegrass content was lower ($P<0.001$) for CC–RG (59%) than WC–RG (67%) pastures, but ingress of invasive grasses (mostly barley grass and smooth brome grass) was similar ($P<0.61$) for all four treatments (8%). Dead material averaged 14% and broadleaf weeds (1%) were mostly chickweed (*Stellaria media*) and dandelion (*Taraxacum officinale*).

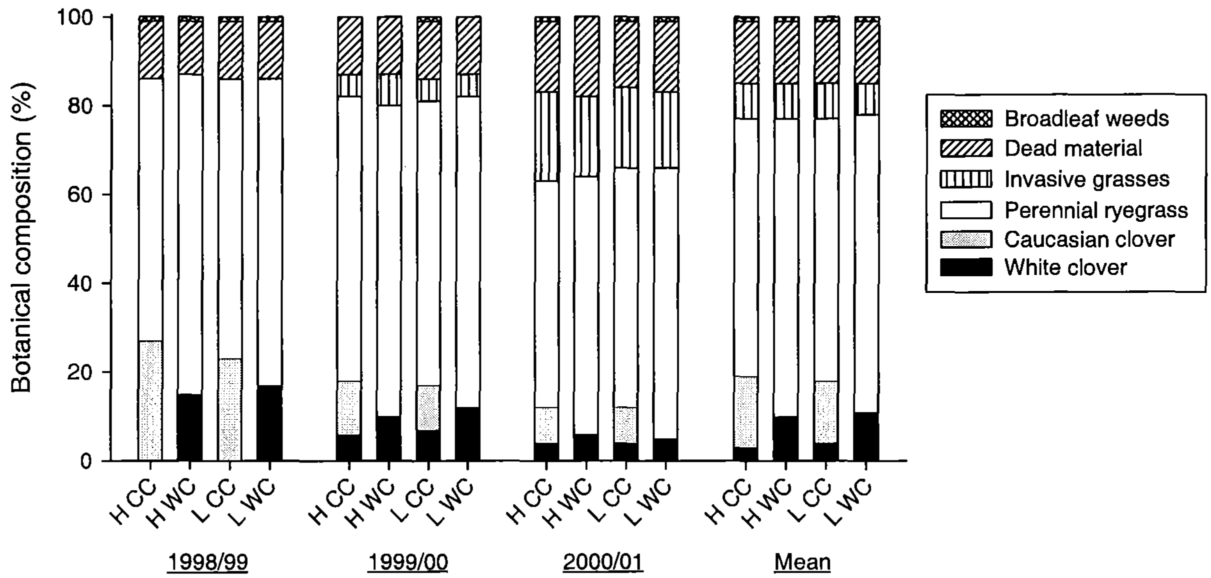

Figure 3.8 Mean botanical composition of Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

Total clover content was consistently greater ($P<0.05$) for CC–RG than WC–RG pastures over the 3 year duration (Figure 3.8). Volunteer white clover in CC–RG pastures was not detected in 1998/99, but averaged ~5% in 1999/00 and 2000/01. There was a decrease over time in total clover content that was associated with the ingress of invasive grasses in all four pastures. Specifically, the annual total clover content for CC–RG and WC–RG pastures in 1998/99 was 25% and 16%, compared with 18% and 11% in 1999/00, and 13% and 5% in 2000/01. Seasonal differences are presented in Figure 3.9.

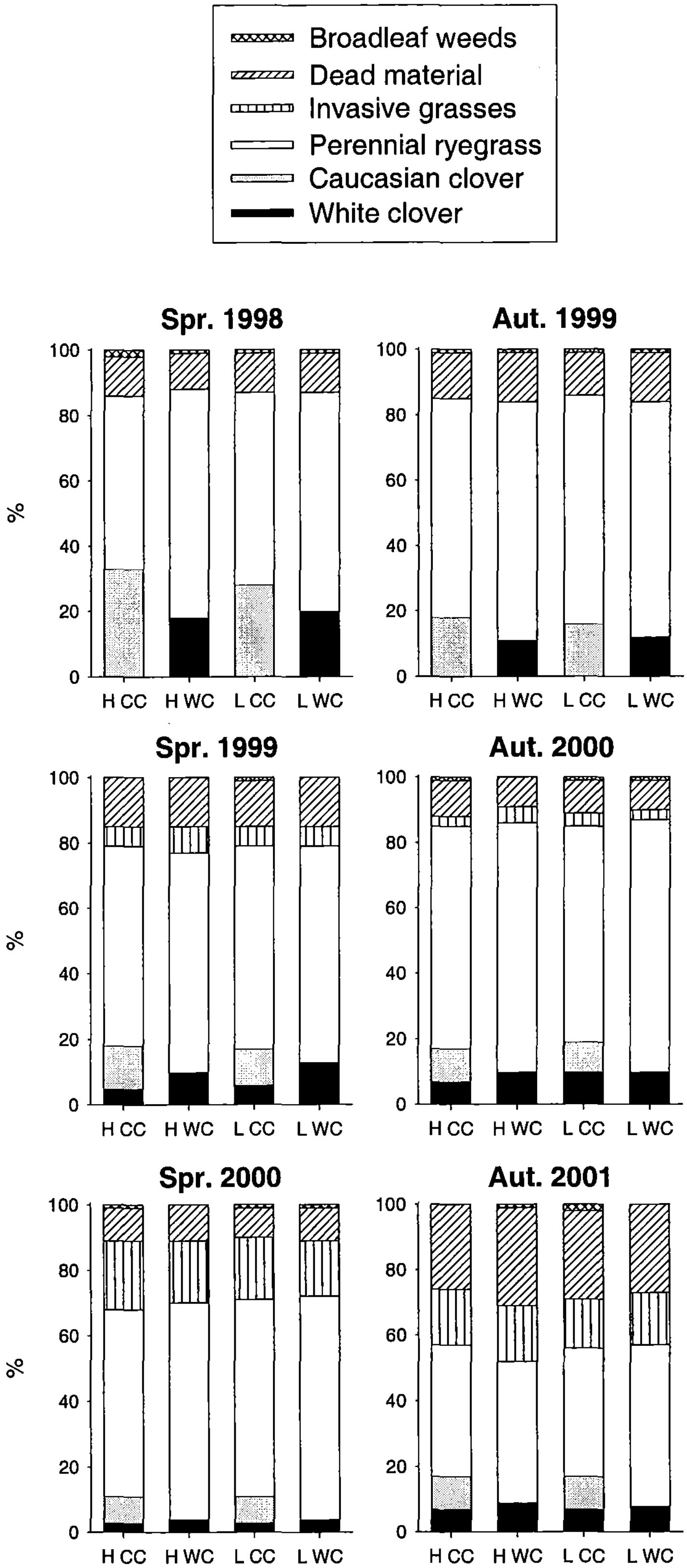

Figure 3.9 Botanical composition of Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions per grazing period in block H17 at Lincoln University, Canterbury, New Zealand.

3.3.6 Nutritive value

The mean 1998–2001 annual CP concentration in clover herbage on offer was 1.5% greater ($P<0.05$) for Caucasian clover (30.2%) than white clover (28.7%), but the ME concentration was similar for both clover species at 12.5 MJ/kg DM (Table 3.8). For ryegrass, the mean annual CP and ME concentrations were similar for both pasture mixtures at 22.7% and 11.5 MJ/kg DM, respectively. There were no significant differences between soil fertility treatments in mean CP and ME concentrations in clover and ryegrass, and nutritive value showed little variation between years and seasons. Additional nutritive value data for each grazing period are given in Appendices 3 and 4.

3.3.7 Mineral concentrations

Over 3 years, the mean P concentration in clover herbage was 0.33–0.35% while S averaged 0.22% under both soil fertility conditions (Table 3.9). However, the mean P concentration in ryegrass herbage in November 2001 was 0.44% for High-F compared with 0.35% ($P<0.01$) for Low-F. Similarly, the mean S concentration in ryegrass herbage was 0.49% for High-F compared with 0.42% ($P<0.01$) for Low-F.

The mean N concentration in Caucasian clover herbage was 4.16% compared with 3.86% ($P<0.01$) for white clover and 3.80% for ryegrass (Table 3.9). Similarly, the mean Ca concentration in Caucasian clover was 0.36% compared with 0.18% ($P<0.05$) for white clover and 0.70% for ryegrass. However, the mean Na concentration in ryegrass was 0.45% compared with 0.38% ($P<0.05$) for white clover and only 0.01% for Caucasian clover. The N concentration in ryegrass was unaffected by clover species.

The macro nutrient concentrations for the two clover species sampled in December 1998, December 1999 and November 2001 are given in Appendix 5. Additional results from analyses of micro nutrient concentrations in clover herbage in December 1998 are given in Appendix 6.

Table 3.8 Mean crude protein (CP) and metabolisable energy (ME) concentrations in clover and ryegrass green leaf herbage on offer in Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998–2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		CP (%)				ME (MJ/kg DM)			
		'98/99	'99/00	'00/01	Mean	'98/99	'99/00	'00/01	Mean
Clover									
High-F	CC	30.4_a	29.9	30.2	30.2	12.5	12.6	12.5	12.5
	WC	28.6_b	29.0	29.1	28.9	12.7	12.5	12.4	12.5
Low-F	CC	29.9_a	29.9	30.5	30.1	12.5	12.6	12.4	12.5
	WC	28.2_b	29.6	27.8	28.5	12.6	12.5	12.5	12.5
s.e.m.		0.10	0.37	0.99	0.42	0.10	0.10	0.04	0.05
P_C		<0.001	0.203	0.143	0.041	0.140	0.373	1.000	1.000
P_F		0.053	0.476	0.648	0.594	1.000	1.000	1.000	1.000
$P_{C \times F}$		1.000	0.476	0.478	0.745	0.182	1.000	0.308	1.000
Ryegrass									
High-F	CC	23.9	23.1	22.5	23.2	11.6	11.5	11.9	11.7
	WC	23.0	22.4	22.5	22.7	11.4	11.3	11.7	11.5
Low-F	CC	23.3	22.9	22.1	22.7	11.4	11.4	11.7	11.5
	WC	22.9	22.2	21.4	22.2	11.5	11.4	11.7	11.6
s.e.m.		0.52	0.34	0.21	0.26	0.08	0.10	0.05	0.05
P_C		0.296	0.143	0.212	0.125	0.584	0.373	0.080	0.215
P_F		0.545	0.645	0.033	0.150	0.584	1.000	0.215	0.638
$P_{C \times F}$		0.724	0.946	0.212	1.000	0.164	0.373	0.215	0.080

Note: Details of soil fertility treatments are given in Section 3.2.3. Subscript C = clover species and F = soil fertility. Within columns, values with the same or no letter subscripts are not significantly different ($\alpha = 0.05$).

Table 3.9 Mean 1998–2001 macro nutrient concentrations in clover leaves plus petioles and ryegrass leaves plus pseudostems for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand.

		Macro nutrient concentrations (%)						
		N	P	S	Mg	Ca	Na	K
Clover[A]								
High-F	CC–RG	4.19_a	0.35_a	0.23	0.29_a	1.37_a	0.01_c	2.77_{bc}
	WC–RG	3.91_b	0.32_b	0.22	0.26_c	1.25_b	0.33_a	2.62_c
Low-F	CC–RG	4.13_a	0.33_b	0.22	0.28_b	1.32_a	0.01_c	2.89_{ab}
	WC–RG	3.80_b	0.33_b	0.22	0.27_{bc}	1.16_c	0.27_b	2.99_a
s.e.m.		0.041	0.002	0.003	0.004	0.015	0.007	0.044
P_C		0.005	0.008	0.071	0.021	0.002	<0.001	0.659
P_F		0.120	0.030	1.000	0.571	0.016	0.029	0.011
$P_{C\times F}$		0.608	0.006	0.116	0.071	0.249	0.029	0.069
Ryegrass[B]								
High-F	CC–RG	3.80	0.43_a	0.51_a	0.21	0.74	0.43	2.64
	WC–RG	3.72	0.45_a	0.48_a	0.22	0.75	0.43	2.80
Low-F	CC–RG	3.86	0.34_b	0.42_b	0.19	0.63	0.37	2.93
	WC–RG	3.83	0.37_b	0.42_b	0.22	0.70	0.60	2.48
s.e.m.		0.131	0.011	0.006	0.008	0.035	0.067	0.161
P_C		0.703	0.104	0.219	0.102	0.291	0.191	0.442
P_F		0.585	0.004	0.001	0.391	0.108	0.452	0.943
$P_{C\times F}$		0.861	0.423	0.103	0.194	0.457	0.191	0.153

Note: [A] Clover data are from samples taken in December 1998, December 1999 and November 2001. [B] Ryegrass data are from samples taken in November 2001 only. Details of soil fertility treatments are given in Section 3.2.3. Subscript C = clover species and F = soil fertility. Within columns and clover and ryegrass, values with the same or no letter subscripts are not significantly different ($\alpha = 0.05$).

3.4 Discussion

In the present study, CC–RG pastures were more productive than WC–RG pastures in terms of sheep LWG per hectare under both high (Olsen P 20 ug/ml, sulphate-S 12 ug/g) and low (Olsen P 11 ug/ml, sulphate-S 7 ug/g) soil fertility conditions.

3.4.1 Effect of clover species

The mean annual sheep LWG per hectare from CC–RG pastures (average 1136 kg) was about 9% greater than that from WC–RG pastures (average 1042 kg) over the 3 years of this study (Table 3.4). This greater sheep LWG per hectare from CC–RG pastures was due to 9% greater ADLWG (Table 3.5) but a similar number of grazing days (Table 3.6) compared with WC–RG pastures. Given that herbage allowance was similar for each treatment (Section 3.2.5) the difference in ADLWG was attributed to botanical composition and its effect on pasture nutritive value. Specifically, the higher ADLWG was probably the result of there being twice as much total clover content (Figure 3.8) of similar or better nutritive value (Table 3.8) in the CC–RG pastures than in the WC–RG pastures.

It seems likely that the greater legume content in CC–RG pastures would have allowed sheep to consume a greater proportion of high quality clover herbage in their diet. At the grazing pressure imposed in this experiment, it is very likely that sheep were unable to select a better diet than the average offered and therefore the two would have been the same. However, LWG is difficult to attribute to any one pasture component as the ratio of species on offer constantly changes due to selective grazing and plant growth rate (Milne *et al.*, 1982). Nevertheless, previous studies have shown that increasing the legume content of pastures increases the proportion of legume in the diet (Clark and Harris, 1985; Gibb and Treacher, 1984; Harris *et al.*, 1998), thereby giving improved animal performance (Askin *et al.*, 1987; Hyslop *et al.*, 2000; Stevens *et al.*, 1993).

The greater legume content in CC–RG pastures shows that Caucasian clover is more productive than white clover in perennial ryegrass-based pastures under both high and low soil fertility conditions with irrigation. This is probably the result of the rhizomatous growth habit of Caucasian clover, which confers a greater competitive ability than the stolons of white clover under these grazed conditions. Recent studies have reported greater legume contents for Caucasian clover than other legumes such as white and red clovers in mixtures with different grass species under well-fertilised, moist soil conditions (Cuomo *et al.*, 2003; Moss *et al.*, 1996; Mourino *et al.*, 2003). In each case, the greater productivity of Caucasian clover has been attributed to its ability to persist and spread due to its underground rhizomes.

Annual ADLWG ranged from 121 to 148 g/head/d across all four treatments (Table 3.5), which is within the expected range for young female sheep grazing on perennial ryegrass-based pasture with a 10 to 30% clover content (Ryan and Widdup, 1997). The use of zero endophyte 'Ruanui' ryegrass and the maintenance of a high proportion of green leaf material (Figure 3.8) in the pastures ensured herbage of high nutritive value (Table 3.8). Animal disorders and production depressions associated with wild-type endophyte from infected ryegrass (Fletcher *et al.*, 1999) were also avoided by the use of zero-endophyte ryegrass. The high nutritive value of Caucasian clover herbage supports findings by other researchers that, like white clover, Caucasian clover is a high forage quality species (Section 2.4). Herbage mineral concentrations were similar in both species, except the Na concentration was lower in Caucasian (0.01 %) than white (0.30%) clover (Table 3.9). This difference is consistent with previous reports of Caucasian clover being a low Na (natrophobe) species, like lucerne and red clover (Edmeades and O'Connor, 2003; Jarvis *et al.*, 1998).

The CC–RG pastures supported a similar number of grazing days (Table 3.6) to the WC–RG pastures due to similar pre-grazing pasture mass (Table 3.7). This is in contrast with Moss *et al.* (1996) who showed that CC–RG pastures had greater total DM yields (15.0 cf. 12.0 t DM/ha/yr) and clover contents (28 cf. 10%) than WC–RG pastures in their fourth year under irrigated lowland conditions. The lack of differences in total DM yield may have been an artefact of measurements made during spring and autumn and not during mid summer (late December/January), when clover production is often greatest (Brougham, 1959), the variable grazing management imposed, and without optimum irrigation. This necessitated the experiment described in Chapter 4 to measure the DM production of the four pasture treatments under a standardised cutting regime using exclosure cages.

3.4.2 Effect of soil fertility

Over 3 years, the annual sheep LWG per hectare from High-F pastures (average 1124 kg) was about 7% greater than that from Low-F pastures (average 1055 kg), regardless of clover species (Table 3.4). This greater sheep LWG per hectare on High-F pastures was due to a 5% increase in the number of grazing days compared with Low-F pastures (Table 3.6) but similar ADLWG (Table 3.5). The number of grazing days on a pasture, or carrying capacity, is a function of its total DM production (Coates and Penning, 2000). In this study, the greater annual number of grazing days on High-F compared with Low-F pastures is attributed to their 5% greater mean annual pre-grazing pasture mass (Table 3.7). These animal and pasture production responses to increased soil fertility were marginal, but were within the range expected for sedimentary soils on lowland farms in New Zealand (Morton *et al.*, 1998; Morton *et al.*, 1999; Sinclair *et al.*, 1997). Thus, Caucasian clover was more productive than white clover under both soil fertility conditions, but both species responded to increased soil fertility (Olsen P and sulphate-S).

The lack of significant difference between the High-F and Low-F treatments in ADLWG (Table 3.5) is principally attributed to the similar botanical composition (Figure 3.8) and herbage nutritive value (Table 3.8) for both soil fertility treatments. Specifically, the similar average total clover contents and CP and ME concentrations in clover herbage suggest that the Olsen P and sulphate-S levels in the Low-F treatments were adequate for the growth of both clover species. The similar clover herbage P (0.34%) and S (0.22%) concentrations from the High-F and Low-F pastures (Table 3.9) support this conclusion. Both clover species were able to meet their P and S requirements under Low-F conditions. Morton and Roberts (2001) also reported similar white clover contents with increased Olsen P in grazed dairy pastures. Also, it is possible that the variable grazing management used in this study, and measurements only in spring and autumn may have limited the ADLWG response.

3.4.3 Seasonal production

The greater annual sheep LWG per hectare from CC–RG pastures was due to greater sheep LWG per hectare than from WC–RG pastures during both spring (September–December/January) and autumn (February–May/June) grazing periods (Figure 3.4). Hoggets in spring produced more live weight than lambs in autumn due to increased ADLWG (Figure 3.5) and number of grazing days (Figure 3.6). However, the greater number of grazing days did not reflect mean pre-grazing pasture mass (Figure 3.7), which was similar for both grazing periods, because pasture allowance was greater for all treatments during spring (2.0–3.0 kg DM/head/d) than autumn (1.5–2.5 kg DM/head/d). Pasture allowance has been shown to influence animal performance (Jagusch *et al.*, 1981; Thompson *et al.*, 1980), and it may have also influenced animal responses in this study.

The CC–RG pastures had greater total clover contents than WC–RG pastures during both spring and autumn (Figure 3.9), which suggests that Caucasian clover is more productive than white clover during both of these periods. However, a more accurate assessment of the seasonal growth patterns of each species is required to determine their relative seasonal responses under these conditions. Furthermore, clover content in pastures is usually at its greatest during summer under irrigated conditions (Brougham, 1959; Rickard and Radcliffe, 1976), but the high live weights of hoggets and shearing requirements in December/January resulted in LWG not being measured during summer in this study. Difficulties in achieving even irrigation also contributed to LWG not being measured during summer. Thus, the annual LWG per hectare measured in this study does not represent the minimum LWG achievable for each year. There is a need to measure the seasonality of production of the two clover species in greater detail.

3.4.4 Botanical composition

The between-year variation in botanical composition of the pastures gives an indication of the relative persistence of the two clover species (Figure 3.8). Specifically, the total clover content of CC–RG and WC–RG pastures declined over the 3 year period, which indicates that both species were declining under high soil N availability and grass competition after the initial legume dominant phase. However, the clover content of CC–RG pastures declined at a slower rate than that of WC–RG pastures, highlighting the ability of Caucasian clover to maintain a greater clover content beyond the initial white clover-dominant phase. The rapid decline in white clover content is consistent with other studies which have reported decreases in white clover plant populations where water status and soil fertility are adequate in the presence of aggressive grasses (e.g. Brock and Hay, 2001). In the present study, the greater persistence of Caucasian clover may be attributed to its rhizomatous growth form allowing it to be more competitive with grasses under rotational grazing. The superior clover content in CC–RG pastures maintained after 4 years justifies the use of Caucasian clover in permanent lowland irrigated pastures.

The total clover content of Caucasian clover pastures in 1999/00 (Year 3) and 2000/01 (Year 4) contained about 5% volunteer white clover (Figure 3.8). This was probably white clover arising from hard seed that survived in the soil for 16 years since the area was last in permanent pasture (R.J. Lucas, pers. comm.). This mixture of the two clover species represents what is most likely to happen even where Caucasian clover is sown without white clover because of the widespread presence of white clover seed in New Zealand pastoral land. Elliot *et al.* (1998) discussed the possibility of poor sociability between Caucasian and white clovers on the basis of rhizobia incompatibility. However, in the present study Caucasian clover, from an agronomic perspective, has appeared to be complementary towards white clover. The compatible association between Caucasian clover and other legume species such as white clover is important in its potential role as the base legume component of permanent pastures.

3.4.5 Irrigation

This experiment has demonstrated the advantage of Caucasian clover over white clover under both high (Olsen P 20 ug/ml, sulphate-S 12 ug/g) and low (Olsen P 11 ug/ml, sulphate-S 7 ug/g) soil fertility conditions with irrigation. However, soil moisture was not always non-limiting. Specifically, rainfall and irrigation in 1998/99 and 2000/01 did not meet PET demand during summer and autumn (Figure 3.1), because of an inability to apply sufficient irrigation water during these times. As a result, the PSWD (Figure 3.2) probably would have limited pasture production. The consequence of this limitation was evident during autumn 2000/01 with a decrease in sheep LWG per hectare (Figure 3.4) and ADLWG (Figure 3.5) due to a reduced number of grazing days (Figure 3.6) and pre-grazing pasture mass (Figure 3.7). This occurred when PSWD had reached ~300 mm (Figure 3.2) but was also partly due to heavy mealy bug damage to all pastures during this time of the year (Section 3.2.8). This meant that soil moisture and biotic factors would have had a confounding affect on the relative performance of the two clover species. Specifically, if Caucasian clover has an advantage over white clover under water stress conditions (Section 2.3.2) then this could have added to its productive advantage. This necessitated the water use experiment in Chapter 5 to isolate temperature and moisture influences on seasonal production of the two clover species.

3.4.6 Establishment of Caucasian clover

In most comparative studies Caucasian clover has received concessions for its slow establishment in the first year (e.g. Watson *et al.*, 1996). Where this has not been considered, the performance of Caucasian clover has often been compromised, usually by excessive grass competition at establishment (Hurst *et al.*, 2000). Excessive grass competition in the first year can delay the time Caucasian clover takes to make a useful contribution to pasture production and quality by 2–4 years (Moss *et al.*, 1996).

In the present experiment, the aim was to provide for successful establishment of Caucasian and white clovers by sowing them as pure species into a seedbed that had been sprayed to control weeds (Section 3.2.2). The clovers were sown in spring which allowed several months of active growth to achieve well-established plants. Following this initial clover establishment period, perennial ryegrass was direct drilled at a moderate sowing rate of 15 kg/ha, which kept the competitive impact low. The influence of slower establishment in Caucasian clover (Section 2.5) was thereby eliminated and both clovers were able to make significant contributions to pasture production as soon as Year 1 (Black, 1998). The CC–RG pastures then matched the sheep LWG per hectare from WC–RG pastures in Year 2 and gave superior LWG per hectare in Years 3 and 4 (Table 3.4). However, lost animal production during the first 1–2 years of establishment is unacceptable on most lowland farms in New Zealand (Taylor and Watson, 1998). Thus, further research is required into the establishment of Caucasian clover, and grass sowing rates and sowing dates for pasture mixtures (Chapters 6 and 7).

3.5 Conclusions

This study showed that Caucasian clover was more productive than white clover in irrigated ryegrass pastures under both low (Olsen P 11 μg/ml, sulphate-S 7 μg/g) and high (Olsen P 20 μg/ml, sulphate-S 12 μg/g) soil fertility conditions. Specific conclusions were:

1. The CC–RG pastures produced ~9% more annual sheep LWG per hectare than WC–RG pastures due to 9% higher ADLWG but similar grazing days, regardless of soil fertility. The greater ADLWG on CC–RG was attributed to greater legume content of similar nutritive value, but similar pre-grazing herbage mass.
2. Both High-F treatments produced ~7% more annual sheep LWG per hectare than Low-F treatments due to 5% more grazing days but similar ADLWG. The similar ADLWG was associated with similar legume content and herbage nutritive value.
3. The CC–RG pastures showed greater productivity than the WC–RG pastures during both spring and autumn, but direct comparisons of seasonal legume performance were limited by variable grazing management and water status. Further research is required to assess the relative seasonal productivity of each species under fully-irrigated and dryland conditions.
4. Caucasian clover showed greater persistence than white clover under rotational grazing in irrigated ryegrass pastures, but the contribution of both legume species to pre-grazing herbage mass decreased over time.
5. Successful establishment of Caucasian clover was critical to achieving increased legume contents. Temporal separation of species achieved this in this experiment but other methods of establishment require examination if Caucasian clover is to be accepted for on-farm use.

Chapter 4

Seasonal dry matter production and nitrogen accumulation rates of Caucasian clover–ryegrass and white clover–ryegrass pastures under high and low fertility soil conditions

4.1 Introduction

In Chapter 3, results showed that CC–RG pastures produced more sheep LWG per hectare than WC–RG pastures under both high (Olsen P 20 µg/ml, sulphate-S 12 µg/g) and low (Olsen P 11 µg/ml, sulphate-S 7 µg/g) soil fertility conditions. This advantage was attributed to the ability of Caucasian clover to maintain a greater clover content in the pasture than white clover. However, direct comparisons of seasonal clover productivity were limited by variable grazing management over seasons.

In New Zealand, the seasonal pattern of pasture production is dependent on total N yield (DM production × % N in the herbage) and is strongly influenced by temperature under adequate moisture conditions (Section 2.3). Radcliffe and Baars (1987) reported that in spring (August–October) pasture production increased by 8 kg DM/ha/°C increase in 10 cm soil temperature from 5.5 to 10 °C, but in autumn (March–May) the response was reduced to 5 kg DM/ha/°C from 7 to 16 °C. Crush (1979) reported that in spring and autumn clover N accumulation and N_2 fixation rates in pastures were correlated with clover DM production rates and temperature. However, N_2 fixation and grass production in pastures are also strongly influenced by soil N supply (Hoglund *et al.*, 1979; Peri *et al.*, 2002a). These relationships were based on WC–RG pastures under standard measurement conditions (Ball *et al.*, 1979; Radcliffe, 1974). They provide a basis for comparisons with other pasture species such as Caucasian clover and different management and soil fertility regimes.

Thus, the initial objective of the research described in this chapter was to use DM production and N accumulation rates for CC–RG and WC–RG pastures to assess the relative seasonal clover performance under High-F and Low-F conditions when temperature (seasonal) varied. Exclusion cages were used to standardise regrowth durations and therefore eliminate any effects of variable grazing management over seasons.

4.2 Materials and methods

4.2.1 Site description

The experiment area was located in block H17 of the Field Service Centre research area at Lincoln University. Sections 3.2.1–3.2.9 describe the site, meteorological conditions, treatments and management.

4.2.2 Measurements

4.2.2.1 Meteorological conditions

Temperature (°C), rainfall (mm), Penman PET (mm) and solar radiation (MJ/m^2/d) were recorded at Broadfields meteorological station (Crop & Food Research Ltd.) located 3 km north of the site (Figures 3.1–3.3).

4.2.2.2 Dry matter production

DM production was measured using 32 exclusion cages (1.0 x 1.4 m) with one cage in each plot. To minimise the coefficient of variation for DM production, each plot was divided into 10 equal 'strata'. The cages were shifted to new pre-trimmed sites in the next stratum after each harvest. The caged areas were trimmed to a uniform height of 30 ± 5 mm at the beginning of 24–37 d regrowth periods (Table 4.1). Regrowth periods were extended to 85–87 d in winter. Total yields at each measurement are given in Appendix 7.

DM production was measured at 6–15 d intervals in the caged areas using a capacitance probe. Measurements began immediately post trimming and finished the day the next regrowth period began. For the final measurement, the probe was calibrated using a paired sample of probe reading and herbage mass from a 0.2 m^2 quadrat cut to 30 ± 5 mm above ground. Sub-samples were taken to determine botanical composition before dry weight was measured.

Data from each harvest was accredited to a standard date (Radcliffe, 1974) at approximately monthly intervals from September to May, with one standard winter date in July (Table 4.1). This enabled years to be treated as repeated measurements. The standard date refers to the mean mid-point date of each regrowth period from 17 September 1998 to 5 June 2001. If the mid-point dates of two regrowth periods occurred in the same month (e.g. Periods 7 and 8 in 1999/00) then data for that month were taken as the mean of both periods.

Table 4.1 Regrowth period start and finish dates and duration from 17 September 1998 to 6 June 2001 in block H17 at Lincoln University, Canterbury, New Zealand.

Season	Regrowth period	Start date	Finish date	Duration (d)	*Standard date
1998/99	1	17 Sep.	14 Oct.	27	27 Sep.
	2	16 Oct.	11 Nov.	26	26 Oct.
	3	11 Nov.	9 Dec.	28	23 Nov.
	4	9 Dec.	12 Jan.	34	20 Dec.
	5	13 Jan.	10 Feb.	28	18 Jan.
	6	10 Feb.	10 Mar.	28	18 Feb.
	7	11 Mar.	7 Apr.	27	23 Mar.
	8	7 Apr.	10 May	33	21 Apr.
	9	4 May	8 Jun.	35	21 May
1999/00	1	11 Jun.	6 Sep.	87	26 Jul.
	2	10 Sep.	6 Oct.	26	27 Sep.
	3	8 Oct.	1 Nov.	24	26 Oct.
	4	3 Nov.	29 Nov.	26	23 Nov.
	5	26 Nov.	20 Dec.	24	20 Dec.
	6	23 Dec.	17 Jan.	25	18 Jan.
	7	19 Jan.	14 Feb.	26	18 Feb.
	8	15 Feb.	14 Mar.	28	18 Feb.
	9	16 Mar.	10 Apr.	25	23 Mar.
	10	13 Apr.	8 May	25	21 Apr.
	11	9 May	8 Jun.	30	21 May
2000/01	1	15 Jun.	8 Sep.	85	26 Jul.
	2	13 Sep.	11 Oct.	28	27 Sep.
	3	13 Oct.	13 Nov.	31	26 Oct.
	4	13 Nov.	11 Dec.	28	23 Nov.
	5	11 Dec.	9 Jan.	29	20 Dec.
	6	9 Jan.	5 Feb.	27	18 Jan.
	7	5 Feb.	5 Mar.	28	18 Feb.
	8	5 Mar.	2 Apr.	28	23 Mar.
	9	2 Apr.	30 Apr.	28	21 Apr.
	10	30 Apr.	6 Jun.	37	21 May

Note: *Standard date refers to the mean mid-point date of each regrowth period from 17 September 1998 to 5 June 2001. If the mid-point date of two regrowth periods occurred on the same month then data for that month were taken as the mean of the two periods (e.g. Periods 7 and 8 in 1999/00).

4.2.2.3 *Botanical composition*

The fresh sub-samples, containing 200–400 pieces, were separated into white clover, Caucasian clover, perennial ryegrass, invasive grasses, broadleaf weeds and dead material before dry weight of each component was measured. Some volunteer white clover was detected in the CC–RG treatments in 1999/00 and 2000/01. The invasive grasses were mostly barley grass and smooth brome grass.

4.2.2.4 *% N and N yield*

The dried sub-samples of clover and grass were retained for determination of % N using the Kjeldahl procedure. Dead, reproductive material and broadleaf weeds were excluded from analyses on the basis that their contribution to N accumulation was likely to be small. Grass and clover samples from each plot were bulked into two replicates of the four treatments representing the eight 'farmlets' of four plots/flock of sheep (i.e. plots 1–16 and 17–32; Chapter 3) and ground in a mill to pass through a 1-mm stainless steel sieve. Analyses were performed by the Animal and Food Sciences Division, Lincoln University. Total, clover and grass N yields were calculated using Equations 2.1 and 2.2.

4.2.2.5 *Clover N_2 fixation*

A N_2-fixation study was carried out in 1998/99 and 1999/00 (Widdup *et al.*, 2001). It used an additional 10 exclusion cages (1.0 x 1.4 m) in five replicates of the two clover treatments under High-F conditions. The caged areas were selected to ensure that adequate populations of the treatment clover species were present. In the CC–RG pastures, areas were selected with minimal amounts of volunteer white clover.

N_2 fixation was assessed using the ^{15}N enrichment method (Ledgard *et al.*, 1985). Beginning in October 1998, the caged areas received labelled ammonium sulphate (40 atom% ^{15}N) at 0.05 g N/m^2 at approximately 8-weekly intervals to label the soil. The caged areas were not moved in 1998/99, but new areas were selected every 8 weeks in 1999/00. At approximately 4-weekly intervals, pasture samples were collected from a 0.2 m^2 quadrat in the centre of each caged area for determination of botanical composition before dry weight was measured. Separate additional sub-samples of clover and grass herbage were dried, finely ground and analysed for total N and ^{15}N concentrations using a mass spectrometer (Anca 20-20 stable isotope analyser).

The proportion of clover N fixed from atmospheric N_2 (P_N) was calculated using Equation 2.3, and the amount of N fixed (kg/ha) in the cut clover herbage was calculated using Equation 2.4.

4.2.3 Statistical analysis

Significant (P<0.05) treatment effects were determined by ANOVA procedures (GenStat, 1997). Years were treated as repeated measurements (Gomez and Gomez, 1984). The variability of annual and seasonal data was expressed as the standard error of the mean (s.e.m.) for the 3 years of measurement, or 2 years (1999/00 and 2000/01) for the standard July date which was not measured in 1998/99 (Table 4.1). Treatment means were compared using Fisher's protected least significant difference (l.s.d.) test whenever the ANOVA indicated that differences among treatments presented P<0.05. Botanical composition percentages were arc-sine transformed as necessary using the method described in Section 3.2.11.

4.3 Results

4.3.1 Seasonal dry matter production rates

4.3.1.1 Total pasture

Total pasture production rate averaged 19 kg DM/ha/d for both High-F treatments from July to September (Figure 4.1a). Total pasture production rates increased rapidly for CC–RG pastures until late October and then increased at a slower rate to a maximum of 94 kg DM/ha/d in November. This rate declined to ~75 kg DM/ha/d in December/January and 54 kg DM/ha/d in March, followed by a faster decline to 15 kg DM/ha/d in May. These production rates were 10–15 kg DM/ha/d higher ($P<0.05$) than for WC–RG pastures from late October to mid February, but similar at the beginning and end of the season. Under Low-F conditions, total pasture production rates were similar between clovers from July to May, and both clovers reached ~81 kg DM/ha/d in late October/November (Figure 4.1b). Total pasture production rates then declined to ~65 kg DM/ha/d in mid December/January followed by a faster decline to average 47 kg DM/ha/d in March and 16 kg DM/ha/d in May.

4.3.1.2 Clover

Clover production rate averaged 1 kg DM/ha/d for both clovers from July to September, under High-F conditions (Figure 4.1a). Clover production rate then increased for CC–RG pastures to a maximum of 29 kg DM/ha/d in November before it decreased to 20–26 kg DM/ha/d in December–February and 11 kg DM/ha/d in March. These clover production rates were 5–16 kg DM/ha/d higher ($P<0.05$) than for WC–RG pastures from September to March. Under Low-F conditions (Figure 4.1b), clover production rates for CC–RG pastures reached a January peak of 24 kg DM/ha/d before they decreased to 11 kg DM/ha/d in March, compared with 11 and 6 kg DM/ha/d ($P<0.05$) for WC–RG pastures.

4.3.1.3 Grass

Grass production rate peaked at ~63 kg DM/ha/d in October but then dropped to 31–60 kg DM/ha/d from December to March for all treatments (Figure 4.1). Grass production rate was similar between clover treatments from December to February under High-F conditions, but

7–9 kg DM/ha/d lower (*P*<0.05) for CC–RG than WC–RG pastures under Low-F conditions. Grass production rate was ~14 kg DM/ha/d for both clover treatments in May.

4.3.2 Botanical composition

Total clover content (sown clover plus volunteer white clover) for CC–RG pastures was greater (*P*<0.001) than for WC–RG pastures from September to April, reaching 35% in December compared with 19% for white clover (Figure 4.2). Volunteer white clover in CC–RG pastures contributed 10% of total DM in January, but averaged 7% or less in other months. Perennial ryegrass was the dominant species, but invasive grasses contributed as much as 24% of total DM in November before declining to 5–9% in January for all treatments. Dead material was 9–13% in December–January and 12–15% in April. Broadleaf weeds never exceeded 6%.

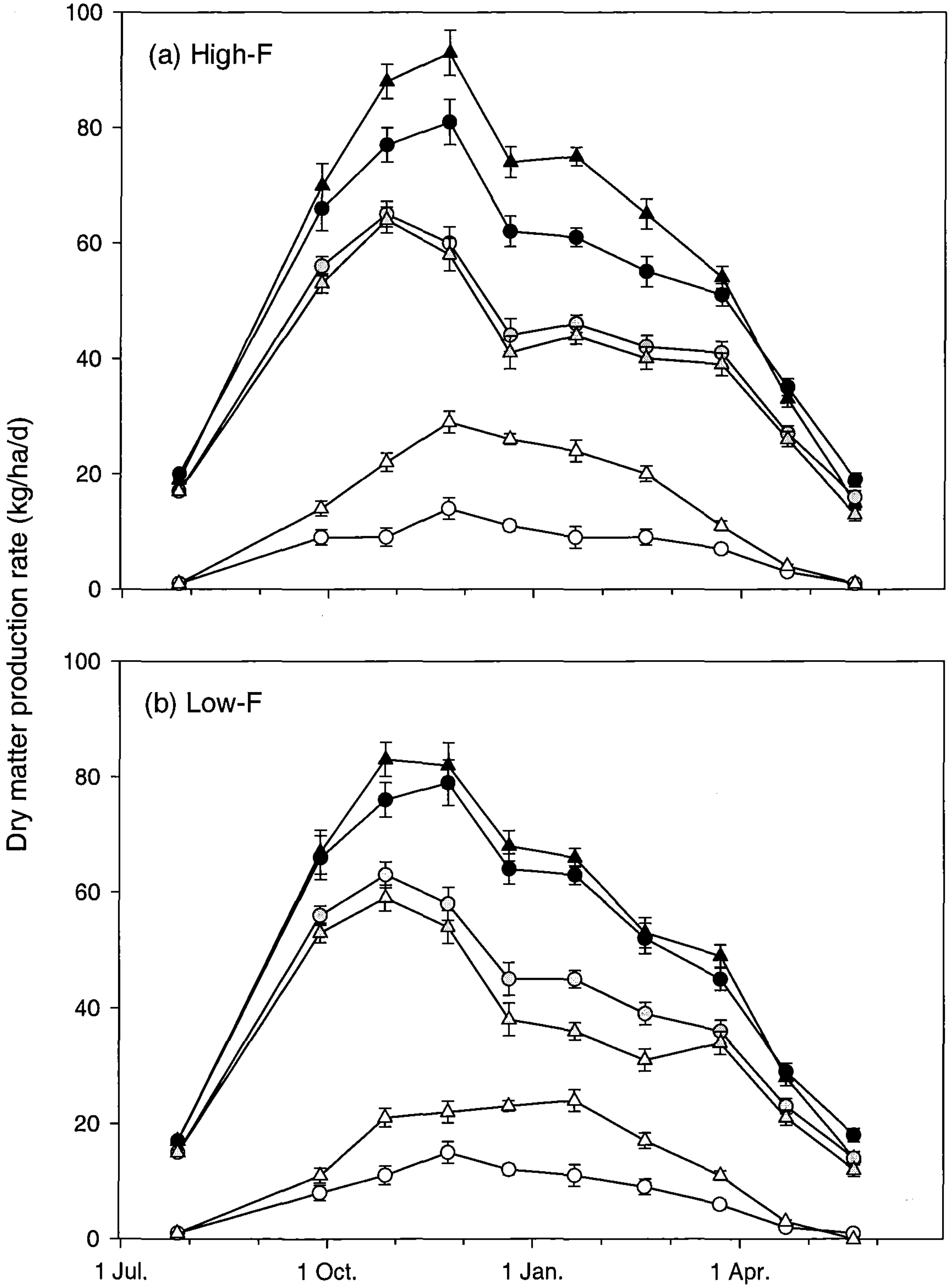

Figure 4.1 Mean 1998–2001 monthly dry matter production rates of total pasture (closed symbols), clover (open symbols) and grass (shaded symbols) components for Caucasian clover-ryegrass (▲) and white clover-ryegrass (●) pastures under high (a) and low (b) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

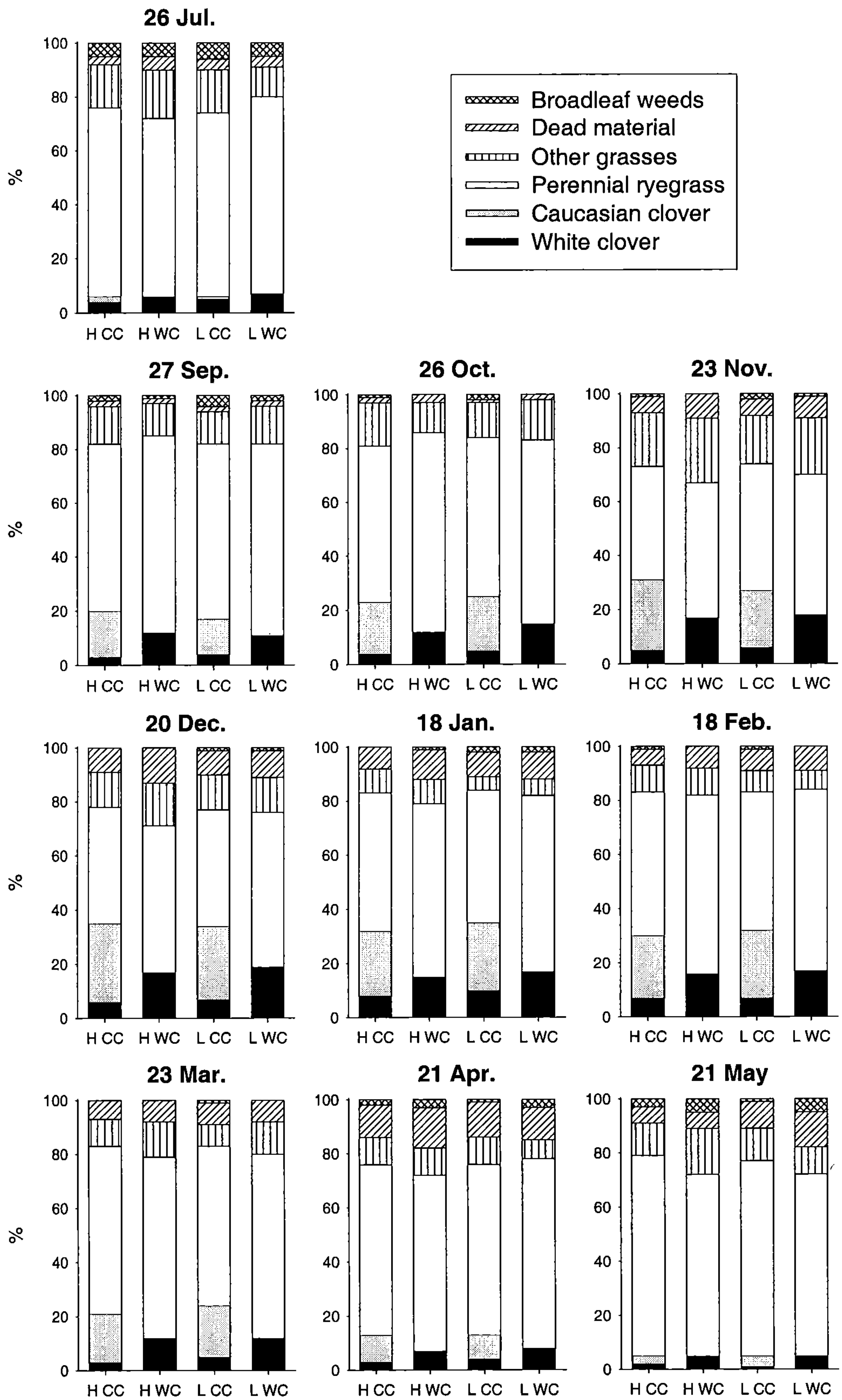

Figure 4.2 Mean 1998–2001 monthly botanical composition of Caucasian clover-ryegrass (CC) and white clover-ryegrass (WC) pastures under high (H) and low (L) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand.

4.3.3 Relationships between dry matter production rate and temperature

4.3.3.1 Total pasture production rate

Early spring (July–September) pasture production rate was linearly related ($R^2 \geq 0.97$; s.e. ≤ 4.4) to mean 10 cm soil temperature between 6.2 and 10.2 °C for all treatments (Figure 4.3a,b). There was a subsequent decline in pasture production rate as mean soil temperature increased to a maximum of 18.9 °C. However, autumn pasture production rate was linearly related ($R^2 \geq 0.95$; s.e. ≤ 4.2) to mean soil temperature between 8.2 and 18.9 °C for all treatments except High-F WC–RG, which declined above 15.9 °C. Early spring pasture production rate increased by ~12.4 kg DM/ha/°C for all treatments, which was three times that achieved in autumn (~4.0 kg DM/ha/°C).

4.3.3.2 Clover production rate

For spring clover production, there was a linear relationship ($R^2 \geq 0.90$; s.e. ≤ 1.8) between mean soil temperature and clover production rate from 6 to 15 °C for all treatments (Figure 4.3c,d). Both clovers started production at the same time (above ~5.3 °C), but for every degree increase in soil temperature, Caucasian clover produced another 3.2 kg DM/ha compared with 1.3 kg DM/ha ($P<0.05$) for white clover under High-F conditions. The response was lower for Caucasian clover under Low-F conditions at 2.5 kg DM/ha/°C compared with 1.5 kg DM/ha/°C ($P<0.07$) for white clover.

For autumn clover production, exponential functions ($R^2 \geq 0.99$) were fitted to clover production rates, which declined as mean soil temperature dropped from 18.9 to 8.2 °C (Figure 4.3c,d). It was notable that the production rate of Caucasian clover was affected more than white clover during this period. For example, Caucasian clover produced ~19 kg DM/ha/d at 18.9 °C compared with 9 kg DM/ha/d for white clover, but both clovers produced less than 1 kg DM/ha/d at 8.2 °C.

4.3.3.3 Grass production rate

Grass production rate was linearly related ($R^2 \geq 0.95$; s.e. ≤ 4.4) to mean soil temperature between 6.2 and 10.2 °C in spring, but declined as mean soil temperature increased to 18.9 °C in summer (Figure 4.3e,f). Autumn grass production rate was linearly related ($R^2 \geq 0.97$; s.e.

≤ 1.0) to mean soil temperature between 8.2 and 15.9 °C, but then declined at 18.9 °C for all treatments. Grass production increased by ~10 kg DM/ha/°C in spring, but decreased by ~3 kg DM/ha/°C in autumn, above a T_b of ~4.2 °C for all treatments.

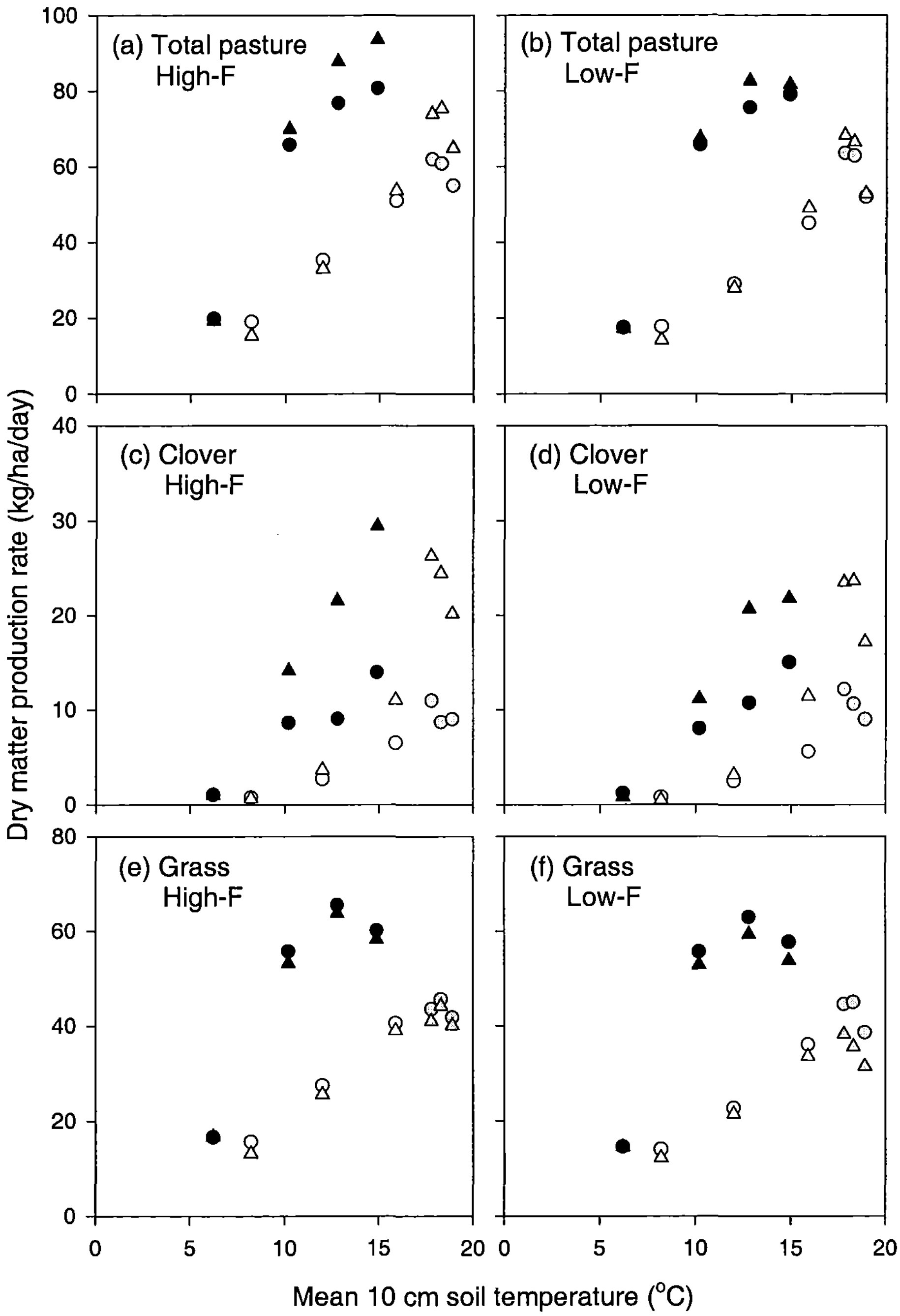

Figure 4.3 Mean 1998–2001 dry matter production rates plotted against mean daily 10 cm soil temperature from July–November (closed), December–February (shaded) and March–May (open) for total pasture (a, b), clover (c, d) and grass (e, f) components from Caucasian clover-ryegrass (▲) and white clover-ryegrass (•) pastures under high (High-F) and low (Low-F) soil fertility conditions at Lincoln University, Canterbury, New Zealand.

4.3.4 Seasonal herbage N % and N accumulation rates

4.3.4.1 Seasonal herbage N%

There was no significant effect of soil fertility on the N% in clover and grass herbage and therefore seasonal data are presented for the two clover species treatments only (Figure 4.4). The N% in clover herbage showed little seasonal variation, ranging between approximately 4.00 and 4.50%, but tended to be lower in spring and summer than in autumn and winter. The N% in Caucasian clover herbage was ~0.16–0.30% higher than in white clover herbage from October to December and from March to April, but similar in other months. There was no significant effect of the two clover species on the N% in grass herbage, which ranged between 3.00 and 3.80% in November and May, respectively.

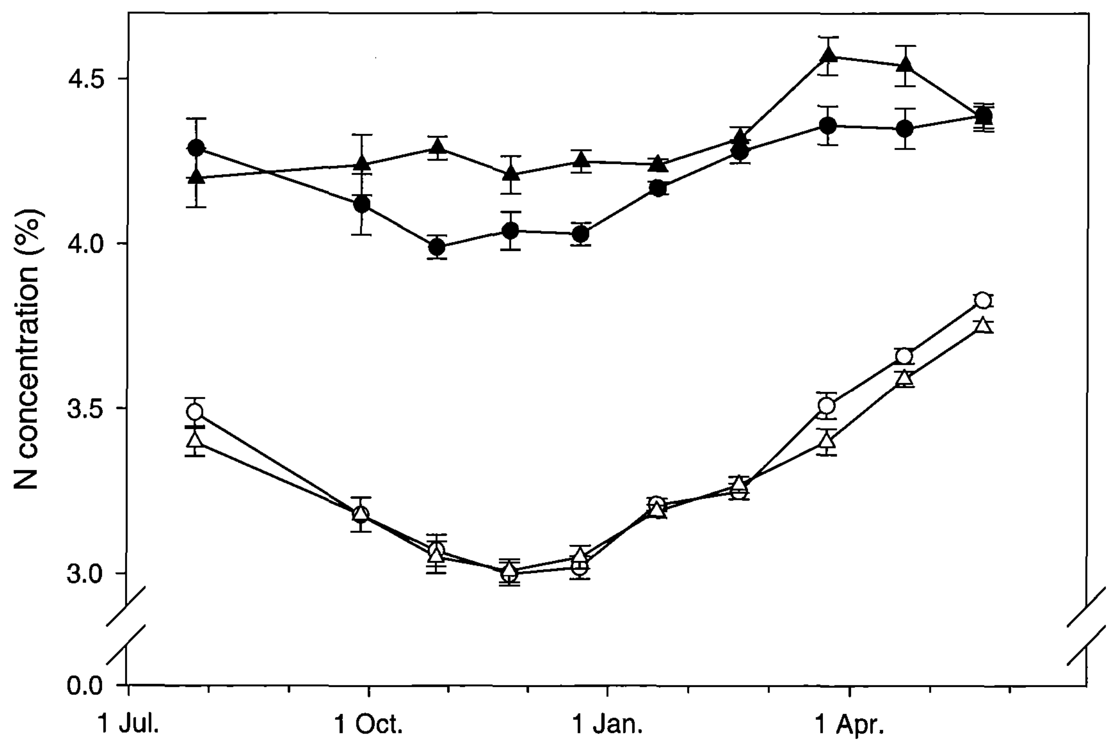

Figure 4.4 Mean N % in clover (▲,●) and grass (Δ,○) herbage for Caucasian clover–ryegrass (▲,Δ) and white clover–ryegrass (●,○) pastures in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

4.3.4.2 Seasonal N accumulation rates

4.3.4.2.1 Total N accumulation rate

Total N accumulation rates in harvested herbage averaged 0.4 kg N/ha/d for both High-F treatments from July to September (Figure 4.5a). Total N accumulation rates increased rapidly for CC–RG pastures until late October and then increased at a slower rate to a maximum of 3.4 kg N/ha/d in November. This rate declined to ~2.6 kg N/ha/d in December/January and 1.9 kg N/ha/d in March, followed by a faster decline to 0.5 kg N/ha/d in May. These N accumulation rates were 0.5–0.7 kg DM/ha/d higher (P<0.05) than for WC–RG pastures from late October to mid February, but similar at the beginning and end of the season. Under Low-F conditions, total N accumulation rates were generally similar between clovers from July to May, and both clovers reached 2.4–2.7 kg N/ha/d in late October/November (Figure 4.5b). Total N accumulation rate was correlated (R^2 = 0.90, P<0.001) with total DM production (Figure 4.1).

4.3.4.2.2 Clover N accumulation rate

Clover N accumulation rate was correlated (R^2 = 0.99, P<0.001) with clover DM production rate (Figures 4.1 and 4.5). Under High-F conditions, the N accumulation rate of Caucasian clover peaked at 1.3 kg N/ha/d in November before declining to between 1.1 and 0.9 kg N/ha/d from December to February. These N accumulation rates were 0.5–0.7 kg N/ha/d higher (P<0.001) than for white clover from November to February. Similar patterns occurred under Low-F conditions where the N accumulation rate of Caucasian clover was 0.9 kg N/ha/d in November and 0.7 kg N/ha/d in February compared with 0.6 and 0.4 kg N/ha/d (P<0.001) respectively for white clover.

4.3.4.2.3 Grass N accumulation rate

A correlation (R^2 > 0.91, P<0.001) occurred between grass N accumulation and DM production rates (Figures 4.1 and 4.5). For all treatments, grass N accumulation rate peaked at ~2.3 kg N/ha/d in November but then dropped to between 1.0 and 1.7 kg N/ha/d from December to March. Grass N accumulation rate was similar between clover treatments under High-F conditions, but 0.2–0.4 kg N/ha/d lower (P<0.05) for Caucasian than white clover treatments under Low-F conditions.

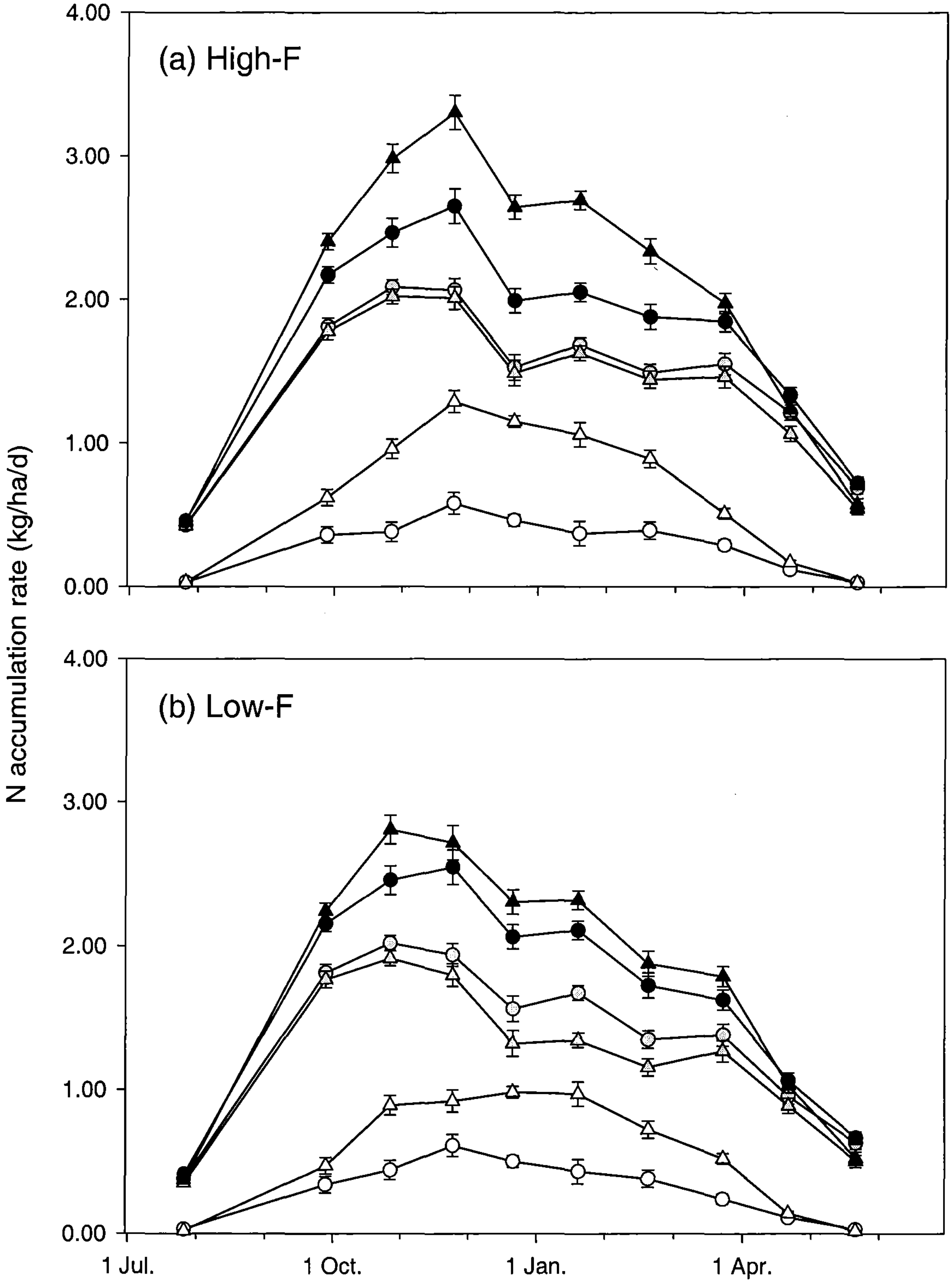

Figure 4.5 N accumulation rate of total pasture (closed symbols), grass (shaded symbols) and legume (open symbols) components for Caucasian clover–ryegrass (▲) and white clover–ryegrass (●) pastures under (a) high and (b) low soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

4.3.5 Seasonal N_2 fixation

4.3.5.1 Proportion of clover N from N_2 fixation (P_N)

The proportion of clover N derived from N_2 fixation (P_N) varied seasonally, and was generally similar for both clover species (Figure 4.6). The P_N values tended to be highest in late spring/summer at about 75% and lowest in late autumn at 45% for both species. There was no significant correlation between P_N and legume DM production rate.

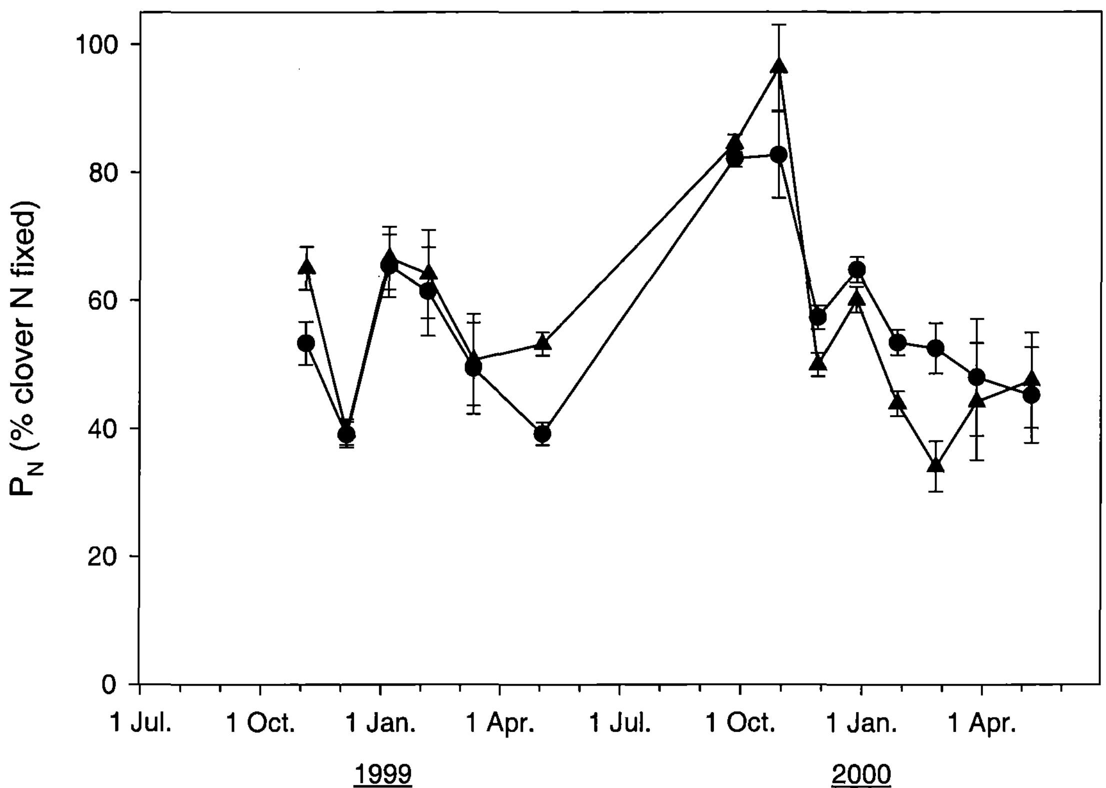

Figure 4.6 Proportion of total clover N derived from N_2 fixation (P_N) for Caucasian clover–ryegrass (▲) and white clover–ryegrass (•) pastures under high soil fertility conditions from 1998–2000 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

4.3.5.2 N_2 fixation rates

There was large seasonal variation in the rate of N_2 fixation by both clover species (Figure 4.7). In 1998/99, the N_2-fixation rate of Caucasian clover was 0.46–1.21 kg N/ha/d between November and March compared with 0.17–0.24 kg N/ha/d for white clover. In 1999/00, the N_2-fixation rate of Caucasian clover was greater in the summer period but similar to white clover in the cooler autumn period. It is likely that the low November/December values in 1999/00 were due to low soil moisture levels, as irrigation was not initiated on the pastures until mid December.

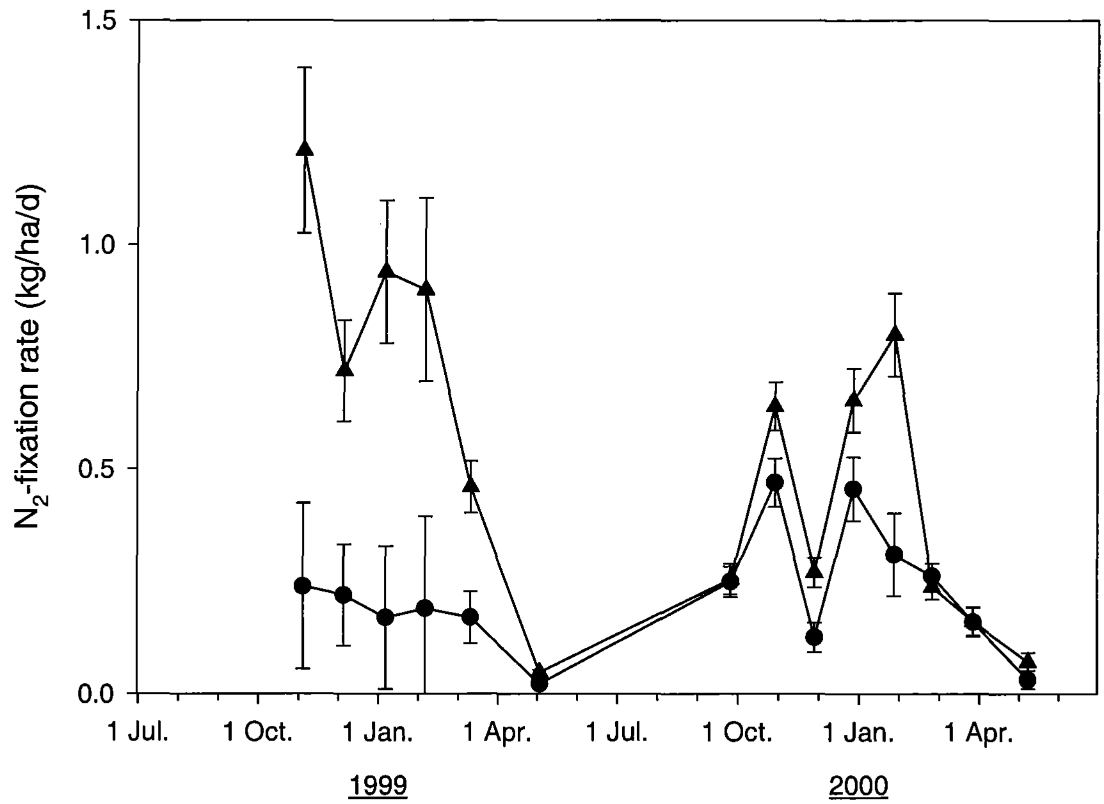

Figure 4.7 Seasonal N_2-fixation rate for Caucasian clover–ryegrass (▲) and white clover–ryegrass (●) pastures under high soil fertility conditions from 1998–2000 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

4.3.6 Annual dry matter yield and composition

4.3.6.1 Total DM yield

Averaged over 3 years, the annual total DM yield was 1.3 t/ha greater ($P<0.05$) for CC–RG than WC–RG pastures under High-F conditions, but similar under Low-F conditions (Table 4.2). Annual total DM yields differed between years, but the treatment effects were consistent in 1998/99 and 2000/01. In 1999/00, total DM yield was similar for both pasture mixtures under High-F conditions, but was 1.1 t/ha greater ($P<0.05$) for CC–RG than WC–RG pastures under Low-F conditions.

4.3.6.2 Clover DM yield

The CC–RG pastures averaged nearly double ($P<0.001$) the annual clover DM yield of WC–RG pastures under Low-F conditions, but the advantage was 0.5 t/ha greater under High-F conditions (Table 4.2). This interaction ($P<0.05$) was not evident for mean annual clover content; CC–RG pastures averaged 25% clover compared with 14% for WC–RG pastures ($P<0.001$) regardless of soil fertility. Annual clover DM yields decreased over the 3 years for both clover species, but the CC–RG pastures consistently produced double ($P<0.001$) the clover DM of WC–RG pastures under both High-F and Low-F conditions in each year, and particularly under High-F conditions in 1998/99. The greater clover yields corresponded to the annual clover content of CC–RG pastures averaging 32% in 1998/99, 22% in 1999/00 and 22% in 2000/01, compared with 20%, 13% and 9% respectively for WC–RG pastures.

4.3.6.3 Grass DM yield

The mean annual grass DM yield was ~1 t/ha lower ($P<0.001$) for CC–RG than WC–RG pastures, and ~1 t/ha greater ($P<0.001$) under High-F than Low-F conditions (Table 4.2). Annual grass DM yields differed between years, but treatment effects were consistent in 1999/00 and 2000/01. In 1998/99, grass DM yield was unaffected by clover species or soil fertility treatments.

Table 4.2 Dry matter (DM) yield of total pasture, clover and grass for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998 to 2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		t/ha			
Total DM yield					
High-F	CC–RG	17.8_a	18.6_a	16.2_a	17.5_a
	WC–RG	15.7_b	18.0_{ab}	15.0_b	16.2_b
Low-F	CC–RG	15.3_b	17.3_b	14.9_b	15.8_{bc}
	WC–RG	15.7_b	16.2_c	14.3_b	15.4_c
s.e.m.		0.47	0.33	0.25	0.25
Clover DM yield					
High-F	CC–RG	5.8_a	3.9_a	3.5_a	4.4_a
	WC–RG	3.0_c	2.1_b	1.3_b	2.1_c
Low-F	CC–RG	4.6_b	4.0_a	3.2_a	3.9_b
	WC–RG	3.1_c	2.3_b	1.3_b	2.2_c
s.e.m.		0.26	0.19	0.18	0.15
Grass DM yield					
High-F	CC–RG	11.2	13.2_b	11.4_b	11.9_b
	WC–RG	11.5	14.2_a	12.5_a	12.8_a
Low-F	CC–RG	10.0	11.5_c	10.4_c	10.7_c
	WC–RG	11.4	12.5_b	11.8_{ab}	11.9_b
s.e.m.		0.42	0.31	0.25	0.24
Clover content					
		%			
High-F	CC–RG	33_a	21_a	22_a	25_a
	WC–RG	19_b	11_c	9_b	13_b
Low-F	CC–RG	30_a	23_a	21_a	25_a
	WC–RG	20_b	14_b	9_b	14_b
s.e.m.		1.3	1.0	1.0	0.8

Note: Details of soil fertility treatments are given in Section 3.2.3. Within columns and variables, values with the same or no letter subscripts are not significantly different (α=0.05) according to Fisher's protected l.s.d. test.

4.3.7 Annual herbage N% and N yield

4.3.7.1 Herbage N%

Mean N% in clover herbage was significantly higher ($P<0.01$) for Caucasian clover than for white clover during 1998/99 and 1999/00, although the mean difference was only ~0.15% (Table 4.3). Mean N% in grass herbage was similar for both CC–RG and WC–RG pastures, and both High-F and Low-F treatments over the 3 years.

Table 4.3 Mean clover and grass herbage N% for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions from 1998/99 to 2000/01 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		%			
Clover herbage N					
High-F	CC–RG	4.78_a	4.19_a	4.23	4.38_a
	WC–RG	4.60_b	4.09_b	4.07	4.25_b
Low-F	CC–RG	4.48_c	4.24_a	4.13	4.30_b
	WC–RG	4.33_d	4.10_b	4.07	4.15_c
s.e.m.		0.018	0.017	0.051	0.016
Grass herbage N					
High-F	CC–RG	3.11	3.19	3.67	3.30
	WC–RG	3.21	3.10	3.68	3.32
Low-F	CC–RG	3.13	3.10	3.50	3.22
	WC–RG	3.04	3.13	3.71	3.30
s.e.m.		0.055	0.014	0.036	0.030

Note: Details of soil fertility treatments are given in Section 3.2.3. Within columns and variables, values with the same or no letter subscripts are not significantly different ($\alpha=0.05$) according to Fisher's protected l.s.d. test.

4.3.7.2 Total N yield

Averaged over 3 years, the annual total N yield (clover plus grass) was 78 kg/ha greater ($P<0.05$) for CC–RG than WC–RG pastures under High-F conditions, but similar under Low-F conditions (Table 4.4). Annual total N yields differed between years, but the treatment effects were consistent.

4.3.7.3 Clover N yield

The CC–RG pastures produced nearly double ($P<0.001$) the average annual clover N yield of WC–RG pastures under Low-F conditions, but the advantage was ~30 kg/ha greater under High-F conditions (Table 4.4). Annual clover N yields decreased over the 3 years for both clover species, but in all years, CC–RG pastures yielded approximately double ($P<0.001$) the clover N yield of WC–RG pastures. This yield advantage was similar under both High-F and Low-F conditions in 1999/00 and 2000/01, but particularly under High-F conditions in 1998/99.

4.3.7.4 Grass N yield

The mean annual grass N yield was ~40 kg/ha lower ($P<0.001$) for CC–RG than WC–RG pastures, and ~40 kg/ha greater ($P<0.001$) under High-F than Low-F conditions (Table 4.4). Annual grass N yields differed between years, but treatment effects were generally consistent.

Table 4.4 Nitrogen (N) yield of clover, grass and total pasture (clover plus grass) for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) under high (High-F) and low (Low-F) soil fertility conditions from 1998/99 to 2000/01 in block H17 at Lincoln University, Canterbury, New Zealand.

		1998/99	1999/00	2000/01	Mean
		kg/ha			
Total N yield					
High-F	CC–RG	621_{a}	556_{a}	570_{a}	582_{a}
	WC–RG	503_{b}	493_{b}	517_{b}	504_{b}
Low-F	CC–RG	514_{b}	491_{b}	498_{b}	501_{bc}
	WC–RG	476_{b}	464_{b}	494_{b}	478_{c}
s.e.m.		16.4	11.7	7.9	8.5
Clover N yield					
High-F	CC–RG	275_{a}	158_{a}	149_{a}	194_{a}
	WC–RG	136_{c}	82_{b}	53_{b}	91_{c}
Low-F	CC–RG	203_{b}	164_{a}	130_{a}	166_{b}
	WC–RG	135_{c}	92_{b}	52_{b}	93_{c}
s.e.m.		11.7	7.8	7.4	6.2
Grass N yield					
High-F	CC–RG	346_{ab}	398_{a}	421_{b}	388_{b}
	WC–RG	367_{a}	410_{a}	464_{a}	414_{a}
Low-F	CC–RG	310_{b}	327_{b}	368_{c}	335_{c}
	WC–RG	342_{ab}	372_{b}	442_{ab}	385_{ab}
s.e.m.		12.3	8.8	9.0	7.1

Note: Details of soil fertility treatments are given in Section 3.2.3. Within columns and variables, values with the same letter subscripts are not significantly different (α=0.05) according to Fisher's protected l.s.d. test.

4.3.8 Annual N_2 fixation

Annual clover DM yield in the N_2-fixation experiment (Widdup *et al.*, 2001) reflected differences reported in Tables 4.2 and 4.4, although in 1998/99 CC–RG produced four times (P<0.01) as much clover DM as WC–RG (Table 4.5). The proportion of total clover N derived from N_2 fixation (P_N) was similar for Caucasian and white clovers. For both clovers, N_2 fixation contributed an average of 57% (52–61%) of their total herbage N over the 2-year period but, compared with white clover, Caucasian clover fixed twice as much N because of its greater productivity. The amount of fixed N in clover herbage varied greatly between years, although in both years, Caucasian clover accumulated more fixed N than white clover.

Table 4.5 Annual clover dry matter (DM) production, nitrogen (N) concentration and N_2 fixation for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high soil fertility conditions during 1998/99 and 1999/00 in block H17 at Lincoln University, Canterbury, New Zealand.

	Clover DM (kg/ha)	Total N (%)	P_N[a] (%)	Fixed N in herbage (kg/ha)
Mean				
CC–RG	4550	4.60	56.6	118
WC–RG	1890	4.57	56.4	50
s.e.m.	392	0.025	0.98	9.8
P	0.009	0.443	0.892	0.008
1998/99				
CC–RG	5330	4.61	55.8	138
WC–RG	1420	4.52	52.0	34
s.e.m.	648	0.031	1.40	15.9
P	0.013	0.092	0.128	0.010
1999/00				
CC–RG	3770	4.59	57.4	98
WC–RG	2370	4.62	60.8	66
s.e.m.	204	0.039	0.94	5.0
P	0.008	0.552	0.062	0.010

Note: [a] Proportion of total clover N from N_2 fixation.

4.4 Discussion

In this experiment, results showed that Caucasian clover was more productive than white clover during spring and summer under low soil fertility conditions (Olsen P 11 μg/ml, sulphate-S 7 μg/g), but the advantage was greatest under high soil fertility conditions (Olsen P 20 μg/ml, sulphate-S 12 μg/g).

4.4.1 Pasture production under high soil fertility conditions

4.4.1.1 Annual production

The annual total clover yield of CC–RG pastures under High-F conditions was double (220%) that of WC–RG pastures, resulting in an 8% increase in annual total pasture production (Table 4.2). This production advantage was consistent with Moss *et al.* (1996) who reported greater total pasture yields and clover contents for CC–RG (15.0 t DM/ha, 28% clover) than WC–RG (12.2 t DM/ha, 10% clover) in their third year with border-dyke irrigation and adequate soil fertility in Canterbury.

The annual total pasture yields for CC–RG (17.5 t DM/ha) and WC–RGG (16.2 t DM/ha) pastures were greater than the annual total yield (10.2 t DM/ha) expected for WC–RG pastures in Canterbury (at Winchmore, ~60 km SW of Lincoln) with irrigation and adequate soil fertility (Rickard and Radcliffe, 1976). The likely reason for this difference is the longer regrowth periods (14 d cf. ~28 d) used in the present experiment compared with those used by Rickard and Radcliffe (1976). Baars (1982) showed the influence of cutting interval on both annual production and the seasonal pattern of production, with 28-d cuts giving 22% more annual DM than 14-d cuts due to the longer regrowth period.

4.4.1.2 Seasonal production

The greater annual clover yield of CC–RG under High-F conditions was due to 5–15 kg DM/ha/d higher clover production rates than WC–RG from September to March (Figure 4.1a). The difference was even greater from October to February with the total pasture production rate differing by 10–15 kg DM/ha/d. These results highlighted the production potential of Caucasian clover in perennial ryegrass pastures during spring and summer with adequate soil fertility and irrigation. The seasonal patterns of DM production offer insights into the timing and extent of environmental factors operating on the two clover species.

4.4.1.2.1 Spring (July–November)

Clover production rates in the mixed grass/clover pastures averaged only 1 kg DM/ha/d from June to September (Figure 4.1a) when temperatures and solar radiation were lowest (Figure 3.3). The relationships between DM production rates and mean daily 10 cm soil temperature showed that clover production rate increased linearly with increasing temperature from July to November (Figure 4.3). This result indicates that temperature was the main factor regulating solar radiation-driven clover production in spring, and that soil moisture (Figure 3.2) was non-limiting at this time.

The magnitude of the clover production response to temperature is of particular interest in late spring when high quality pasture for peak lactation from ewes and cows is required to offset the decline in grass nutritive value. In the present experiment, the greater clover contents in spring for CC–RG (Figure 4.2) were due to a greater response of Caucasian clover (3.2 kg DM/ha/°C) than white clover (1.3 kg DM/ha/°C) to increasing soil temperatures from 6 to 15 °C (Figure 4.3c). This means that for every degree increase in spring soil temperature from 6 to 15 °C Caucasian clover is expected to produce double the amount of white clover in perennial ryegrass pastures under High-F conditions. The explanation for this difference is related to the physiology of Caucasian clover and white clover that will be described in Chapter 5.

The total pasture production response to temperature from 5.5 to 10 °C in early spring (July–September) was similar (~12 kg DM/ha/°C) for CC–RG and WC–RG pastures (Figure 4.3a), highlighting the rapid growth of ryegrass at this time. However, grass production rates slowed at temperatures above 10 °C (Figure 4.3c). This enabled Caucasian clover to compete successfully with ryegrass and increase total pasture production. It is likely that this decline in grass production coincided with late flowering, when reproductive tillers mature and few vegetative tillers remain, giving slower leaf expansion (Anslow, 1966) and regrowth. Radcliffe and Baars (1987) reported that this reduction occurs even if soil moisture and N are non-limiting.

For both pasture mixtures, the total pasture production response to temperature in spring (12 kg DM/ha/°C) was greater than the response of 8 kg DM/ha/°C from 5.5 to 10 °C predicted for WC–RG by Radcliffe and Baars (1987). It is likely that this difference was due to the longer cutting interval used in this experiment (28–30 d) compared with that used (~14 d) in the series of standardised experiments (Radcliffe, 1974) summarised by Radcliffe and Baars (1987). This difference highlights a limitation of using published seasonal pasture production data for future comparisons of different species or management regimes.

4.4.1.2.2 Summer (December–February)

For both pasture mixtures, clover production rates decreased in summer (Figure 4.3), when soil temperatures were increasing from 15 to 18 °C (Figure 3.3). This suggests that despite best efforts with irrigation, DM production was probably limited by soil moisture at this time (Figure 3.2). However, in summer Caucasian clover had the greatest advantage over white clover, with 10–16 kg DM/ha/d higher clover and total pasture production rates (Figure 4.1), and twice as much total clover content (Figure 4.2). These differences highlight the superior productivity of Caucasian clover during summer and suggest that it will be most suited to environments where increased summer clover production is important and summer droughts are rare.

The likelihood that soil moisture was limiting for clover growth in summer means that the influence of temperature on seasonal DM production could not be isolated. It is possible that Caucasian clover was more productive because of its greater response to temperature (Section 4.4.1.2.1). However, it is also possible that Caucasian clover had an ability to remain productive under moisture-limiting conditions. This leads to the hypothesis that Caucasian clover uses water more efficiently than white clover, or is able to access soil moisture from deeper in the profile due to its perennial taproot (Forde *et al.*, 1989). Other legume species with deep taproot systems such as lucerne can extract water from a greater depth than shallow rooting species such as ryegrass and white clover (Evans, 1978). These ideas also led to the experiment described in Chapter 5 which was designed to isolate the influences of temperature and water status on seasonal production by the two clover species.

Thus, Caucasian clover has potential to increase total pasture production rates during spring and summer. Total pasture production rates for CC–RG ranged from 55–90 kg DM/ha/d from

September to March (Figure 4.1), which was higher than the range of values reported for irrigated WC–RG pastures (33–56 kg DM/ha/d) at Winchmore, Canterbury (Rickard and Radcliffe, 1976). This indicates that Caucasian clover would be suitable for increasing summer pasture production and quality in lowland pastoral systems with adequate P and S fertiliser.

4.4.1.2.3 Autumn (March–May)

Total pasture, clover and grass production rates all responded to changes in mean soil temperature, but production rates were higher in spring than at similar temperatures in autumn (Figure 4.3). Radcliffe and Baars (1987) also reported a difference in spring and autumn production rate responses to temperature for WC–RG pasture. Peacock (1975) attributed this difference to changes in reproductive development and assimilate partitioning in perennial ryegrass during spring and autumn. In the present experiment, inadequate soil moisture (Figure 3.2) and insect pest damage (Section 3.2.8) during autumn would have exaggerated this difference. However, for both pasture mixtures the total production rate response (4–5 kg DM/ha/°C) to temperature from 16 to 8 °C (Figure 4.3) was similar to that reported for white clover-ryegrass in autumn by Radcliffe and Baars (1987).

The decline in clover DM production rate with decreasing temperatures was greater for Caucasian (from 11 to 1 kg DM/ha/d) than white (from 7 to 1 kg DM/ha/d) clover (Figure 4.3c). Caucasian clover may therefore be more sensitive to decreasing temperatures in autumn than white clover. This is an issue related to the growth and development of the two clover species that will also be dealt with in Chapter 5. The difference suggests that the greater autumn clover content described for CC–RG over WC–RG pastures in Chapter 3 was due to higher clover production rates in late summer (when 10 cm soil temperatures were above 15 °C) rather than in autumn. This implies that the superiority of Caucasian clover over white clover may not be as great during autumn as it is during spring and summer.

4.4.2 Pasture production under low soil fertility conditions

In this experiment, the Low-F treatment had a mean Olsen P of 11 μg/ml and a mean sulphate-S level of 7 μg/g.

4.4.2.1 Annual yield

The annual clover yield of CC–RG under Low-F conditions (3.9 t DM/ha) was 74% greater than that of WC–RG pastures (2.3 t DM/ha). This production advantage was ~0.5 t DM/ha less than that under High-F conditions, and contributed to a total pasture yield which was similar to that of WC–RG under Low-F conditions (average 15.6 t DM/ha). The annual white clover yield was similar under both High-F and Low-F conditions, and both white clover treatments had similar P and S concentrations in their herbage (Section 3.3.7). This indicates that soil fertility was non-limiting for white clover production under Low-F conditions. Thus, the seasonal pattern of white clover production under Low-F conditions needs no further analysis. But it is useful to understand when soil fertility (i.e. P and S) became limiting for Caucasian clover production.

4.4.2.2 Seasonal yield

It is possible to establish when soil fertility became limiting for Caucasian clover production by comparing seasonal production rates under High-F and Low-F conditions. The clover production rates showed an obvious difference only in November when the production rate of Low-F Caucasian clover was ~7 kg DM/ha/d (~20%) less than the corresponding treatment under High-F conditions (Figure 4.1). At this time temperature (Figure 3.3) and soil moisture (Figure 3.2) were probably ideal for clover production. But the influence of temperature and soil moisture probably overrode the limiting effect of soil fertility on Caucasian clover production during the rest of the season.

Thus, temperature and soil moisture appear to be the main factors affecting the seasonal production of Caucasian and white clovers in intensive lowland conditions. The experiment described in Chapter 5 was therefore designed to focus on the effects of temperature and moisture on the seasonal production of the two species under non-limiting soil fertility (pH, P, K, and S) conditions.

4.4.3 Nitrogen yield and N_2 fixation

4.4.3.1 Annual N yield and N_2 fixation

Caucasian clover and white clover had similar proportions of total N (Table 4.3) and fixed N_2 in their herbage (Table 4.5), which indicates that both clovers have a similar ability to fix N_2 in the presence of perennial ryegrass, under irrigated and high soil fertility conditions. The N yields of total pasture, clover, and grass (Table 4.4) were directly related to the amounts of DM produced (Table 4.2). Similarly, the amount of N_2 fixed per hectare was directly related to the amount of clover DM produced by the two clover species (Table 4.5). Caucasian clover produced four times more DM than white clover in 1998/99 (5330 cf. 1420 kg DM/ha) and this was associated with four times the amount of N fixed (138 cf. 34 kg N/ha). In 1999/00, Caucasian clover produced 50% more DM than white clover, and the annual amount of N fixed in the CC–RG pastures was therefore 50% greater than that in the WC–RG pastures. The N_2 fixation study was only conducted on the high fertility soil treatments, but given the small difference in clover DM production and N yield between the two treatments it is unlikely that soil fertility had much influence on the N_2-fixing ability of each clover species.

In the N_2 fixation study, the CC–RG pastures had exceptionally high clover contents in 1998/99, which can be partly explained by the management of the N_2 fixation plots. Four weekly cutting with no excreta return for 8 months accentuated the clover component and depressed the grass component. In 1999/00, ^{15}N was applied to a new caged area every 8 weeks, a management allowing greater exposure of plots to normal grazing and excreta return. As a result, the clover contents in 1999/00 were lower than in 1998/99 (Widdup *et al.*, 2001).

4.4.3.2 Seasonal N yield and N_2 fixation

The N accumulation rates were closely correlated with DM production rates, which in turn were correlated with temperature during spring and autumn, and were possibly influenced by moisture stress during summer. Therefore, any environmental factor regulating clover DM production would affect N yield in a similar way. This was demonstrated by the seasonal patterns of N accumulation which were similar to those for seasonal DM production (Figures 4.1 and 4.5).

The mean N% in clover herbage was similar for both species and was relatively constant throughout the year at about 4.0 to 4.5%, indicating that N was non-limiting for clover growth (Figure 4.4). In contrast, the mean N% in grass herbage decreased from ~3.6% in May to ~3.0% in November, which suggests that grass production may have been limited by N stress during this time of year (Figure 4.3). Although the mean N% in clover herbage was relatively constant over time, both clover species showed variation in P_N across seasons of the year (Figure 4.6). The P_N was highest during late spring/summer (~65-95%) and lowest during autumn (~45%) for both clover species. Ledgard *et al.* (2001) attributed lower P_N levels during autumn and early spring to low temperatures reducing clover production, coupled with increased soil mineral N during these periods. Hoglund *et al.* (1979) reported that the level of soil mineral N has a major modifying effect on clover N_2 fixation – as soil mineral N availability increases, N_2 fixation decreases. High P_N occurred during late spring/summer when clover production rates increased with increasing temperatures and soil mineral N levels are normally lower owing to rapid growth and high N uptake by grass (Widdup *et al.*, 2001). Consequently, the seasonal pattern of N_2 fixation rates for each species varied greatly over seasons, but was generally greater for Caucasian clover than white clover during spring and summer (Figure 4.7), due to Caucasian clovers greater production rate during those seasons.

4.5 Conclusions

In the absence of variable grazing management over seasons, this experiment has shown that Caucasian clover was more productive than white clover on low fertility soils (Olsen P 11 µg/ml, sulphate-S 7 µg/g), but particularly on high fertility soils (Olsen P 20 µg/ml, sulphate-S 12 µg/g) during spring and summer. Specific conclusions were:

1. The CC–RG pastures on High-F soil had 0.5–0.7 kg DM/ha/d (20–30%) greater total N accumulation rates than WC–RG pastures due to 0.5–0.7 kg N/ha/d (120–190%) greater clover N accumulation rates from October to February.
2. There was less advantage for CC–RG pastures on Low-F soils with total N accumulation rates that were similar to those for WC–RG pastures, but clover N accumulation for CC–RG pastures was still 50–120% higher than WC–RG pastures from October to February.
3. Clover N accumulation rate was closely correlated with clover DM production rate, which was correlated with temperature during spring and autumn. For every degree increase in spring 10 cm soil temperature, Caucasian clover produced another 3.2 kg DM/ha compared with 1.3 kg DM/ha. In autumn, Caucasian clover produced ~19 kg DM/ha/d at 18.9 °C compared with 9 kg DM/ha/d for white clover, but both clovers produced less than 1 kg DM/ha/d at 8.2 °C. Despite irrigation, summer clover DM production was probably influenced by soil moisture which was less than optimal.
4. Both clovers had similar proportions of fixed N in their herbage and therefore similar N-fixing ability in irrigated ryegrass pastures on high fertility soil. The amount of N fixed per hectare by Caucasian and white clovers was directly related to clover DM production.

In summary, the results presented in this chapter have shown that Caucasian clover would be expected to be more productive than white clover in spring and summer, and the advantage would be greatest with adequate levels of available P and S. However, there is a lack of information on the mechanisms that contributed to the seasonal production advantage. In the following chapter the reasons for these differences in production between the two clovers are investigated.

Chapter 5

Water use efficiency, growth and development of Caucasian and white clovers under irrigated and dryland conditions

5.1 Introduction

In Chapter 4, results showed that Caucasian clover was more productive than white clover in irrigated ryegrass pastures particularly during summer. This advantage was partly attributed to a greater response to warm temperatures, but may also have been due to its greater productivity under water stress conditions. Other studies have reported Caucasian clover to be more productive than white clover in mixtures with different perennial grass species under summer dry conditions (Black and Lucas, 2000; Watson *et al.*, 1998). These agronomic results suggest that Caucasian clover is potentially more drought tolerant than white clover, but there is limited information about the mechanisms responsible for such results.

Understanding plant growth and development responses to temperature and water status is necessary to understand differences in seasonal production between species. Specifically, leaf photosynthesis rate (as an indicator of potential growth) and leaf appearance rate per shoot apex (vegetative development) are important components of seasonal clover production that are strongly regulated by temperature (Haycock, 1981; Woledge and Dennis, 1982) and water status (Guobin and Kemp, 1992; Johns, 1978). Furthermore, there are no comparisons of the water use efficiency (WUE) and water extraction patterns of Caucasian and white clovers (Section 2.6.2). White clover is an inefficient user of water compared with grasses (Johns and Lazenby, 1973b), and has a shallow nodal root system compared with the deep taproot system of Caucasian clover (Section 2.2).

Thus, the initial objective of the research described in this chapter was to compare the DM production, WUE, and water extraction patterns of Caucasian and white clovers when temperature and moisture differed, but fertility was non-limiting. The second objective was to relate any differences in seasonal DM production to the growth (leaf photosynthesis rate) and development (leaf appearance rate) characteristics of each species. To do this, monocultures of the two clover species were established under fully irrigated and dryland (rain-fed) conditions.

5.2 Materials and methods

5.2.1 Site description

5.2.1.1 Location

The experimental area was located on flat land in Iversen Field block 10 of the Field Service Centre research area at Lincoln University, Canterbury, New Zealand.

5.2.1.2 Soil

The soil is a Wakanui silt loam (Udic Ustochrept, USDA Soil Taxonomy). General descriptions (Cox, 1978) state that Wakanui silt loam soils have 0.18–0.35 m of uniform silt loam topsoil overlaying variable textural layers that range from fine silt to loamy sand or sand in texture. Wakanui soils are imperfectly drained and display strong mottling below 0.7 m which indicates periods of water logging (Watt and Burgham, 1992). The available water holding capacity ranges from 120 to 180 mm/m depth (Watt and Burgham, 1992; Webb *et al.*, 2000). For the same soil in Iversen Field block 8 (~30 m from the site), the total water holding capacity was measured as 270–350 mm/m depth, of which ~50% is expected to be available (Brown, 1999; Inch, 1998). Depths to the underlying gravels were reported to be more than 2.3 m.

5.2.1.3 Meteorological conditions

5.2.1.3.1 Rainfall, evapo-transpiration and irrigation

The experiment was conducted over 2 years (Years 1 and 2). The first year (2000/01) was the driest with annual rainfall (485 mm from 1 July–30 June) 28% below the long-term mean (LTM) of 670 mm, but Penman PET was similar to the LTM of 1060 mm (Table 5.1). However, in the second year (2001/02) annual rainfall (699 mm) was 4% above the LTM and PET was 10% below the LTM. The amount of irrigation water applied was 507 mm in 2000/01 and 251 mm in 2001/02. Details of irrigation management are given in Section 5.2.3.

Table 5.1 Rainfall, Penman potential evapo-transpiration (PET) and the amount and timing of irrigation water applied for two years (1 July–30 June) and the long-term mean (LTM) for Iversen 10 at Lincoln University, Canterbury, New Zealand.

Year	Rainfall (mm)	PET (mm)	Irrigation (mm)	Timing of irrigation
2000/01	485	1048	507	21 Nov.–20 Apr.
2001/02	699	953	299*	19 Nov.–24 Mar.
LTM	670	1060		

Note: Rainfall and PET data were obtained from Broadfields meteorological station located 3 km north of the site. *Includes 48 mm of irrigation water applied from 22 August and 20 September because rainfall had not recharged soil water completely in winter. At this time, 166 mm was also applied to the dryland treatment.

Monthly rainfall was variable over the two seasons, ranging from ~6 mm/month in February–April 2001 to 125 mm in January 2002 (Figure 5.1). Monthly PET followed a similar pattern in each season, increasing from a minimum of ~30 mm in July to a maximum of between 120 and 170 mm in December before decreasing to a minimum again in June. Mean daily vapour pressure followed a similar pattern, ranging from ~8 Pa in June/July to ~14 Pa in January/February.

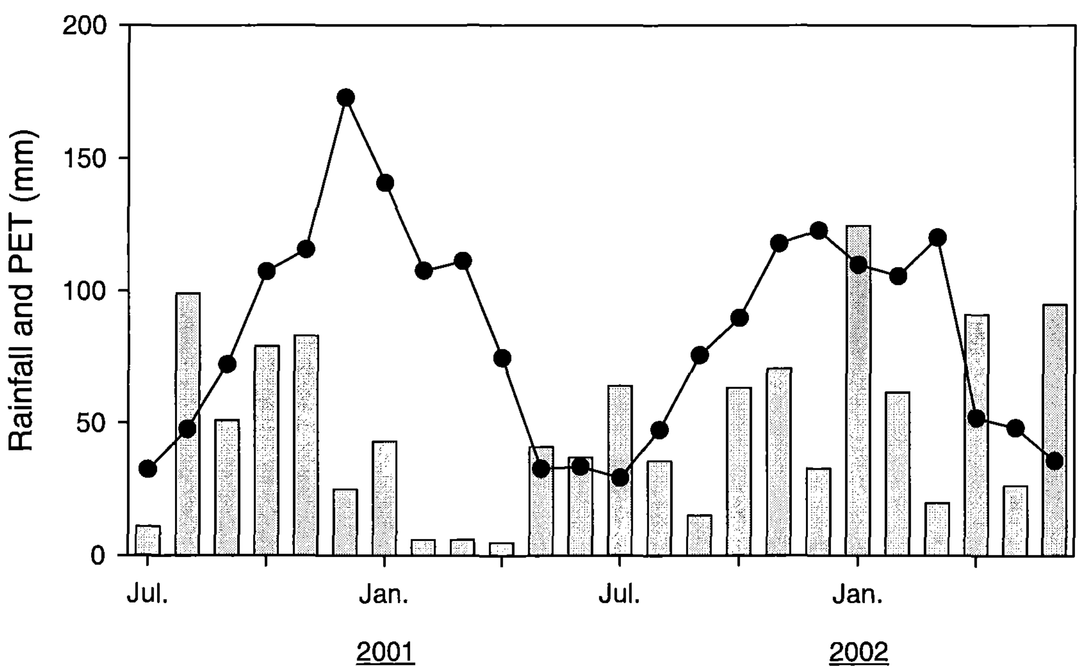

Figure 5.1 Monthly rainfall (bars) and Penman potential evapo-transpiration (PET, •) from 1 July 2000 to 30 June 2002. Data were obtained from Broadfields meteorological station (3 km north of the site), Canterbury, New Zealand.

5.2.1.3.2 Temperature and solar radiation

The mean daily air temperature followed a similar pattern in each season, ranging from 4–8 °C in June–August to 15–17 °C in February (Figure 5.2). The mean daily total solar radiation followed a similar pattern each season, increasing from a minimum of 4–5 MJ/m²/d in June/July to a maximum of 20–25 MJ/m²/d in December.

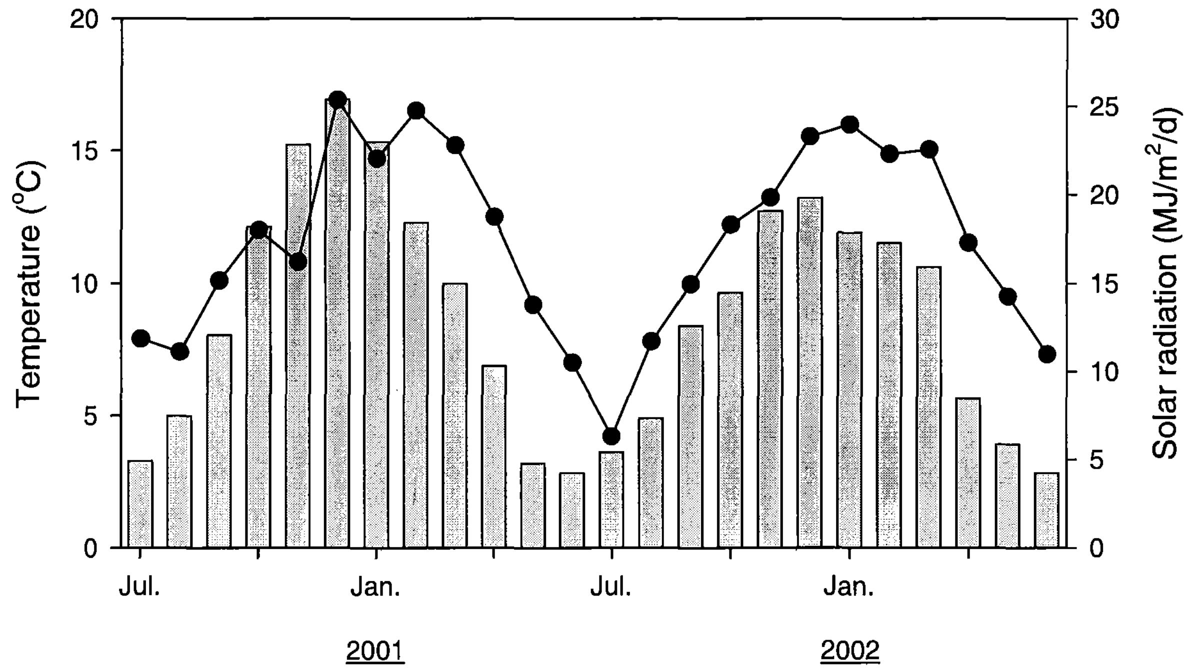

Figure 5.2 Mean daily air temperature (•) and mean daily solar radiation (bars) from 1 July 2000 to 30 June 2002. Temperature was recorded at the site. Solar radiation data were obtained from Broadfields meteorological station (3 km north of the site), Canterbury, New Zealand.

5.2.2 Pasture establishment

The experiment used a split-plot design with three replicates of fully irrigated or dryland treatments as main plots. Sub-plots (4.2 x 6.0 m) were Caucasian and white clovers.

The experimental area had previously contained a kabuli chickpea (*Cicer arietinum* L.) irrigation experiment (Anwar, 2001), but had been in perennial ryegrass pasture for 8 months prior to this experiment. Prior to sowing, the experimental area was cultivated using conventional methods to produce a firm and fine textured seedbed. Sulphur superphosphate (8% P, 19% S) at 250 kg/ha was applied during cultivation, based on soil test results in July 1999 (Table 5.2). Further tests in May 2001 indicated that soil fertility was adequate for maximum clover growth.

Table 5.2 Soil test (0–150 mm) results in July 1999 and May 2001 for Iversen 10 at Lincoln University, Canterbury, New Zealand.

	pH (H_2O)	Olsen P (μg/ml)	SO4-S (μg/g)	Ca^{++}	K^+	Mg^{++}	Na^+
				(meq/100 g)			
July 1999	6.2	27	5	7.2	1.0	1.0	0.14
May 2001	6.2	25	8	8.2	1.1	1.1	0.24

Note: Soil samples were analysed using Ministry of Agriculture and Fisheries Quick Test (MAF QT) procedures.

On 9 November 1999, 'Grasslands Demand' white clover (2 kg/ha) and hexaploid 'Endura' Caucasian clover (8 kg/ha) were sown as monocultures. Clover seed was lime-coated and inoculated with the *R. trifolii* strain CC275e for white clover and ICC148 for Caucasian clover. Sub-plots were drilled with an Øyjoord cone seeder at 150 mm row spacing and a target depth of 15 mm.

By 25 January 2000, both clovers had established only 40 plants /m^2, or equivalent to about 23% of sown seed established. 1000 coated seed weights were 4.40 g for Caucasian clover and 1.20 g for white clover. Thus, on 17 February 2000 more Caucasian (16 kg/ha) and white (4 kg/ha) clover seed was broadcast during grazing in an attempt to increase the clover populations and accelerate the establishment of complete canopies.

5.2.3 Irrigation

In the first year (1999/00) all plots were irrigated to prevent a soil water deficit of 25 mm to 0.5 m soil depth to ensure clovers established. Irrigation treatments were imposed from 2000/01 onward. Irrigation water was applied post-grazing at a rate of 8–10 mm/h using a T-tape irrigation system. Water was applied with the aim of preventing the development of a soil water deficit of >100 mm to 1.5 m depth using a soil water budget:

Equation 5.1 $$A = \sum PET - (R + I)$$

where the amount of water required (A) is equal to the difference between PET (mm/d) and rainfall (R) plus irrigation (I) in the previous period.

The amount of water applied (Table 5.1) was measured using a flow rate meter. From 22 August to 20 September 2001 dryland treatments received 166 mm and irrigated treatments received 48 mm of irrigation water because rainfall had not recharged soil water completely in winter. This returned the Caucasian clover treatments to 15–20 mm below field capacity (FC) and the white clover treatments to 35–45 mm below FC. Details of the amount and timing of irrigation are given in Appendix 8.

5.2.4 Grazing management

In 1999/00 the experimental area was grazed with young sheep at 4–6 week intervals when 1500–2500 kg DM/ha had accumulated. From then on the experimental area was grazed for 4–14 d duration, at 28–37 d intervals. Regrowth periods were extended to 45–70 d in the cool May–September period. Details of the timing and duration of regrowth periods are given in Appendix 9.

5.2.5 Weeds and insect pests

Weed control included the herbicide '2,4-DB' (a.i. 2,4-DB at 2.4 kg a.i./ha) on 23 December 1999 and regular hand weeding of mainly wireweed (*Polygonum aviculare*). In September each season, the herbicide 'Gallant' (a.i. haloxyfop at 250 g a.i./ha) was sprayed to control mainly annual poa (*Poa* spp.) and barley grass. On 24 November 1999, the insecticide

'Chlor-P 480EC' (a.i. chlorpyrifos at 240 g a.i./ha) was sprayed to control greasy cutworm (*Agrotis ipsilon*) that caused ~20% loss of emerged clover plants in all sub-plots.

5.2.6 Measurements

5.2.6.1 Meteorological conditions

Air temperature (°C) was recorded from a single sensor placed in a Stevenson screen adjacent on site. Temperatures were recorded every 5 minutes and integrated and logged every hour with a data logger (DT100 data-taker) to determine daily mean temperatures. Rainfall (mm), PET (mm) and solar radiation (MJ/m^2/d) were recorded at Broadfields meteorological station (Crop & Food Research Ltd.) located 3 km north of the site in Iversen 10.

5.2.6.2 Dry matter production

DM production was measured at 7–15 d intervals in 2000/01 and 2001/02 using a capacitance probe. Measurements began immediately post grazing and finished on the day of the next grazing. For the final measurement, the probe was calibrated using a paired sample method (Section 4.2.2.2). This involved 0.2 m^2 quadrat cuts to 30 ± 5 mm above ground from each sub-plot. Sub-samples were taken to determine botanical composition before dry weight was measured.

5.2.6.3 Botanical composition and bare ground percentage

The fresh sub-samples, containing 200–400 pieces, were separated into white clover, Caucasian clover, weeds and dead material before dry weight of each component was measured. The percentage of bare ground was determined pre-grazing using point analysis at 100 points in each sub-plot.

5.2.6.4 Volumetric soil water content

In August 2000, one aluminium neutron probe access tube was installed in the centre of each plot to 2.3 m depth. Also at this time, two stainless steel time domain reflectometer rods (0.2 m length) were installed within 0.2 m of each neutron probe access tube.

Volumetric soil water content (θ, mm^3/mm^3) was measured at 7–14 d intervals in 2000/01 and 2001/02. Measurements were made in the top 0.2 m of soil using time domain reflectometry (Trace System, Soil Moisture Equipment, PO Box 30025, Santa Barbara, California, 93105, USA). Measurements from 0.25 m were made at 0.10 m intervals to a depth of 1.25 m, and at 0.20 m intervals to 2.25 m, using a neutron probe (Troxler Electronic Industries Inc., Research Triangle Park, North Carolina, 27709, USA).

5.2.6.5 Soil water calculations

5.2.6.5.1 Total soil water content

The θ of each layer was multiplied by the layer depth (10 or 20 mm) to calculate the soil water content (SWC, mm) in each layer, which was then summed for all layers down to 1.7 m depth to determine the total soil water content (TSWC, mm) of the profile.

5.2.6.5.2 Soil water deficit

Soil water deficit (SWD) represents the difference between TSWC at FC and TSWC calculated from soil moisture measurements at 7–14 d intervals. Daily changes in SWD were calculated using a soil water balance:

Equation 5.2 $SWD = SWD_i + \text{daily } WU - (R + I)$

where SWDi is the SWD on the previous day and daily WU is the actual evapo-transpiration (AET) or water use (mm/d, Equation 5.4). R and I are daily rainfall and irrigation, respectively. The maximum soil water deficit (MSWD) for each growth season was calculated from these data.

5.2.6.5.3 Field capacity

Field capacity was estimated as TSWC measured on 1 September 2000 (580 mm to 1.7 m depth). This equates to ~340 mm/m depth which is consistent with the maximum FC of 350 mm/m depth reported by Inch (1998) and Brown (1999) for the same soil in an adjacent field.

5.2.6.5.4 Water use

Water use (WU) was calculated for each period between soil water measurements using a soil water balance:

Equation 5.3 $WU = R + I - (TSWC_F - TSWC_S)$

where $TSWC_S$ and $TSWC_F$ are the actual total soil water contents at the start and finish of each measurement period, respectively. R and I are the sum of rainfall and irrigation for each measurement period, respectively. This equation assumes soil water movement (e.g. drainage) and runoff are zero.

Daily WU within each measurement period was then calculated as:

Equation 5.4 Daily WU = (WU/PET)*Daily PET

where WU is the calculated water use (Equation 5.3) and PET is Penman potential evapo-transpiration for the corresponding measurement period. Daily PET is PET on the day of calculation.

5.2.6.5.5 Water use efficiency

Water use efficiency (WUE) was calculated in 2001/02, when both species had developed complete canopies, by dividing DM yields from each regrowth period by WU for the corresponding period (Equation 2.6).

5.2.6.5.6 Soil water extraction

Soil water extraction was calculated as the difference between the upper and lower limits of extraction for each soil layer and the total soil profile.

5.2.6.6 Leaf photosynthesis

Leaf photosynthesis rate was measured on a random sample of three youngest fully expanded intact leaves. All measurements were taken at midday ± 1 h on cloudless sunny days. Net photosynthesis rate was measured using a Li-Cor LI-6400 portable photosynthesis system (Lincoln, Nebraska, USA) at a maximum light intensity of 1700 ± 100 μmol $CO_2/m^2/s$ PPDF. There were 84 measurements taken in 2000/01 and 2001/02. Of these measurements, 40 were used to determine responses to air temperature (soil moisture non-limiting) and 44 were used for responses to soil moisture (temperature non-limiting). Non-limiting soil moisture photosynthesis values were those measured from irrigated treatments. Non-limiting temperature photosynthesis values were initially measured at the reported optimum temperature of 24 °C for white clover growth (Mitchell, 1956), but the temperature range was then extended to include values that achieved measured photosynthesis rates ≥90% of the highest value recorded.

5.2.6.7 Leaf appearance

Leaf appearance was measured at 5–7 d intervals during each regrowth period in 2000/01. Emerged leaves were counted on five shoot apices in each sub-plot. New shoots were tagged post-grazing.

5.2.6.8 Thermal time calculation

Thermal time (Tt), expressed in degree-days (°Cd) was calculated daily using the method described by Arnold and Monteith (1974) using Equation 2.5. Leaf appearance was regressed as a function of thermal time to calculate the phyllochron (°Cd/leaf).

The suitability of the thermal time concept is dependent on the use of an appropriate T_b (Bonhomme, 2000; Brown, 2003). An incorrect T_b causes systematic variation or increased dispersion in development rates when they are related to thermal time over a range of temperatures. This concept was used to determine a suitable T_b for Caucasian and white clovers. Initially, a T_b of 0 °C was used for both species (Moot *et al.*, 2000). The T_b was then increased by increments of 0.5 °C to minimise the coefficient of variation for the relationship between leaf appearance rate and thermal time over the growing season.

5.2.7 Statistical analysis

Data were analysed using ANOVA procedures (GenStat, 1997). Treatment means were compared using Fisher's protected l.s.d. test whenever the ANOVA indicated that differences among treatments presented $P<0.05$. Botanical composition percentages were arc-sine transformed as necessary using the method described in Section 3.2.11. The response of leaf photosynthesis rate to temperature and water status was described using a two-piece 'broken stick' model (Draper and Smith, 1998).

5.3 Results

5.3.1 Soil water deficit (SWD)

Under irrigated conditions, SWD was maintained at less than 130 mm for both species in 2000/01 and 2001/02 (Figure 5.3). Under dryland conditions, the SWD for white clover increased to a maximum of 330 mm on 25 April 2001, which was 60 mm greater ($P<0.05$) than for Caucasian clover. Irrigation water applied to both dryland treatments from 22 August to 20 September 2001 to reduce the SWD for the following year (2001/02). This returned the SWD for white clover to 48 mm and Caucasian clover to 24 mm in mid September. The 2001/02 season had more rainfall than the previous season (Figure 5.1), and the SWD for white clover increased to a maximum of 200 mm on 22 March 2002, which was 30 mm greater than for Caucasian clover.

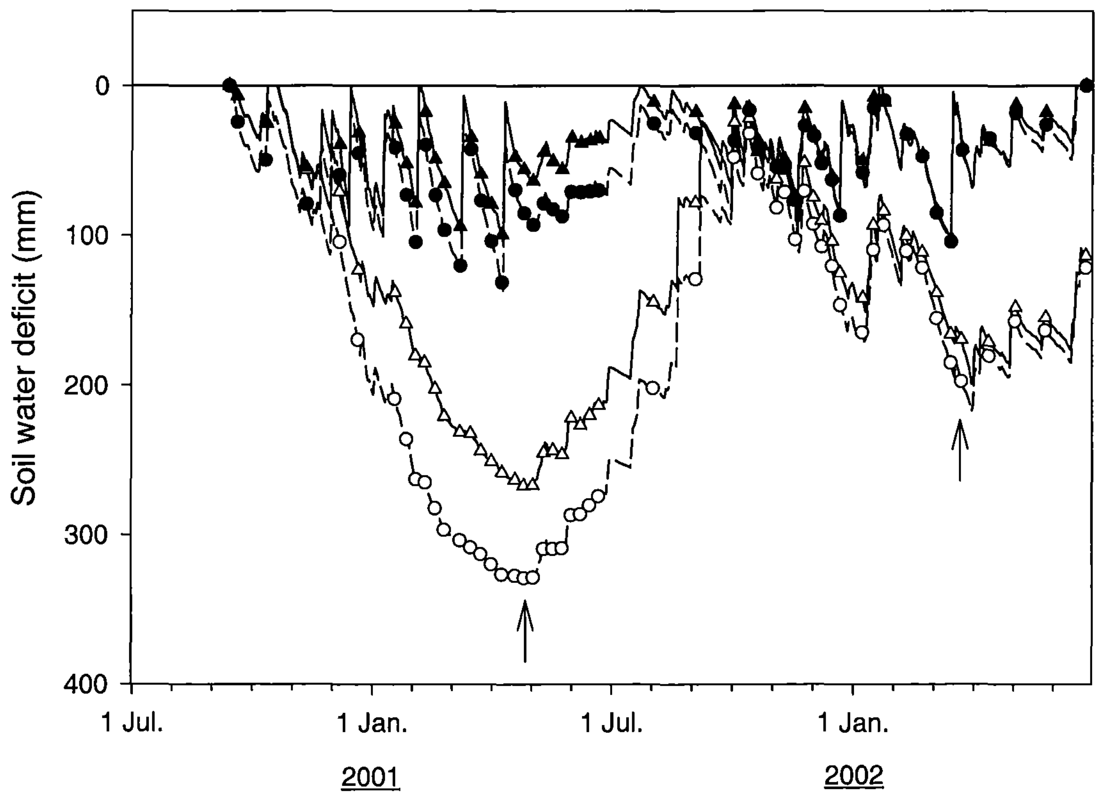

Figure 5.3 Soil water deficit to 1.7 m depth for Caucasian (▲) and white (•) clovers under irrigated (closed symbols) and dryland (open symbols) conditions from 1 July 2000 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Arrows indicate the time of maximum soil water deficit.

5.3.2 Annual dry matter production, botanical composition, bare ground percentage and water use

5.3.2.1 Clover dry matter production

The effect of irrigation on DM production of the two clover species differed between seasons (Table 5.3). In 2000/01, there was an interaction (P<0.01) between species and irrigation. Under irrigated conditions, white clover produced 11 980 kg DM/ha, which was 5920 kg DM/ha more than Caucasian clover. Under dryland conditions, white clover yield was 7020 kg DM/ha, but the yield advantage over Caucasian clover was less at 3390 kg DM/ha.

In the following year (2001/02), irrigated Caucasian clover out-yielded white clover with 11 860 kg DM/ha, which was 2530 kg DM/ha greater (P<0.05) than that of irrigated white clover (Table 5.3). Caucasian clover production was 2510 kg DM/ha less (P<0.01) under dryland conditions at 9350 kg DM/ha, but the yield advantage (P<0.05) over white clover was similar at 2380 kg DM/ha.

5.3.2.2 Botanical composition and bare ground percentage

In 2000/01, clover content averaged 92% in white clover treatments compared with 61–69% in Caucasian clover treatments (Table 5.3). The remainder was broadleaf weeds (Section 5.2.5). Irrigated white clover had complete ground cover compared with 5% bare-ground for irrigated Caucasian clover, and both dryland treatments averaged 15% bare-ground. In 2001/02, there was no bare ground in any treatment and average clover content was greater than 82%.

5.3.2.3 Water use

In 2000/01, annual water use was ~320 mm greater (P<0.01) under irrigated than dryland conditions for both species, and white clover water use averaged 60 mm more (P<0.001) than for Caucasian clover (Table 5.3). In 2001/02, the two irrigated treatments used ~910 mm of water compared with ~740 mm (P<0.001) for both dryland treatments.

Table 5.3 Dry matter (DM) production and mean botanical composition, bare ground percentage, and water use for Caucasian (CC) and white (WC) clovers under irrigated and dryland conditions in 2000/01 and 2001/02 in Iversen 10 at Lincoln University, Canterbury, New Zealand.

		Clover DM (kg/ha)	Total DM (kg/ha)	Clover content (%)	Bare ground (%)	Water use (mm)
2000/01						
Irrigated	CC	6060_b	8650_b	61_c	5_b	977_a
	WC	$11\,980_a$	$12\,690_a$	93_a	1_c	1012_a
Dryland	CC	3630_c	5080_c	69_b	16_a	643_b
	WC	7020_b	7540_b	90_a	13_a	706_b
s.e.m.		369	450	0.9	1.0	12.5
P_I		0.017	0.019	0.102	0.006	0.003
P_C		<0.001	<0.001	<0.001	0.047	<0.001
$P_{I\times C}$		0.003	0.014	0.004	0.444	0.055
2001/02						
Irrigated	CC	$11\,860_a$	$12\,680_a$	89	0	918_a
	WC	9330_b	$10\,050_b$	91	0	908_a
Dryland	CC	9350_b	$10\,380_b$	83	0	758_b
	WC	6970_c	8350_c	84	0	721_b
s.e.m.		445	254	3.1	–	7.4
P_I		0.007	0.021	0.078	–	<0.001
P_C		0.015	<0.001	0.753	–	0.060
$P_{I\times C}$		0.905	0.219	0.937	–	0.219

Note: Details of irrigation treatments are given in Section 5.2.3. Subscript I = irrigation, C = clover species. Within columns, values with the same or no letter subscript are not significantly different (α=0.05).

5.3.3 Seasonal dry matter production in 2001/02

Species comparisons of seasonal DM production were concentrated on the 2001/02 season when both clover treatments had developed complete canopies. Data from the 2000/01 season are given in Appendices 10 and 11.

5.3.3.1 Clover dry matter accumulation

Under irrigated conditions, both clovers began production at the same time in the first spring regrowth period in 2001/02, and by 20 September both clovers had produced about 0.5 t DM/ha (Figure 5.4a). Caucasian clover yields then increased to 2.3–3.1 t DM/ha for each harvest from 4 October to 10 March (Periods 2–5), before declining to 0.6 t DM/ha on 23 April (Period 6) and 0.1 t DM/ha on 25 June (Period 7). Caucasian clover yields were 0.6–0.9 t DM/ha greater ($P<0.05$) than white clover from October to March, but 0.4 t DM/ha less ($P<0.05$) in April.

Under dryland conditions, Caucasian clover yields were 0.2–1.1 t DM/ha lower ($P<0.05$) than those under irrigated conditions from 4 October to 23 April (Periods 2–6), but were similar at the beginning and end of the season (Figure 5.4b). Caucasian clover yields were 0.4–0.9 t DM/ha greater ($P<0.01$) than white clover from October to March (Periods 2–5), but both clovers yielded less than 0.4 t DM/ha in April.

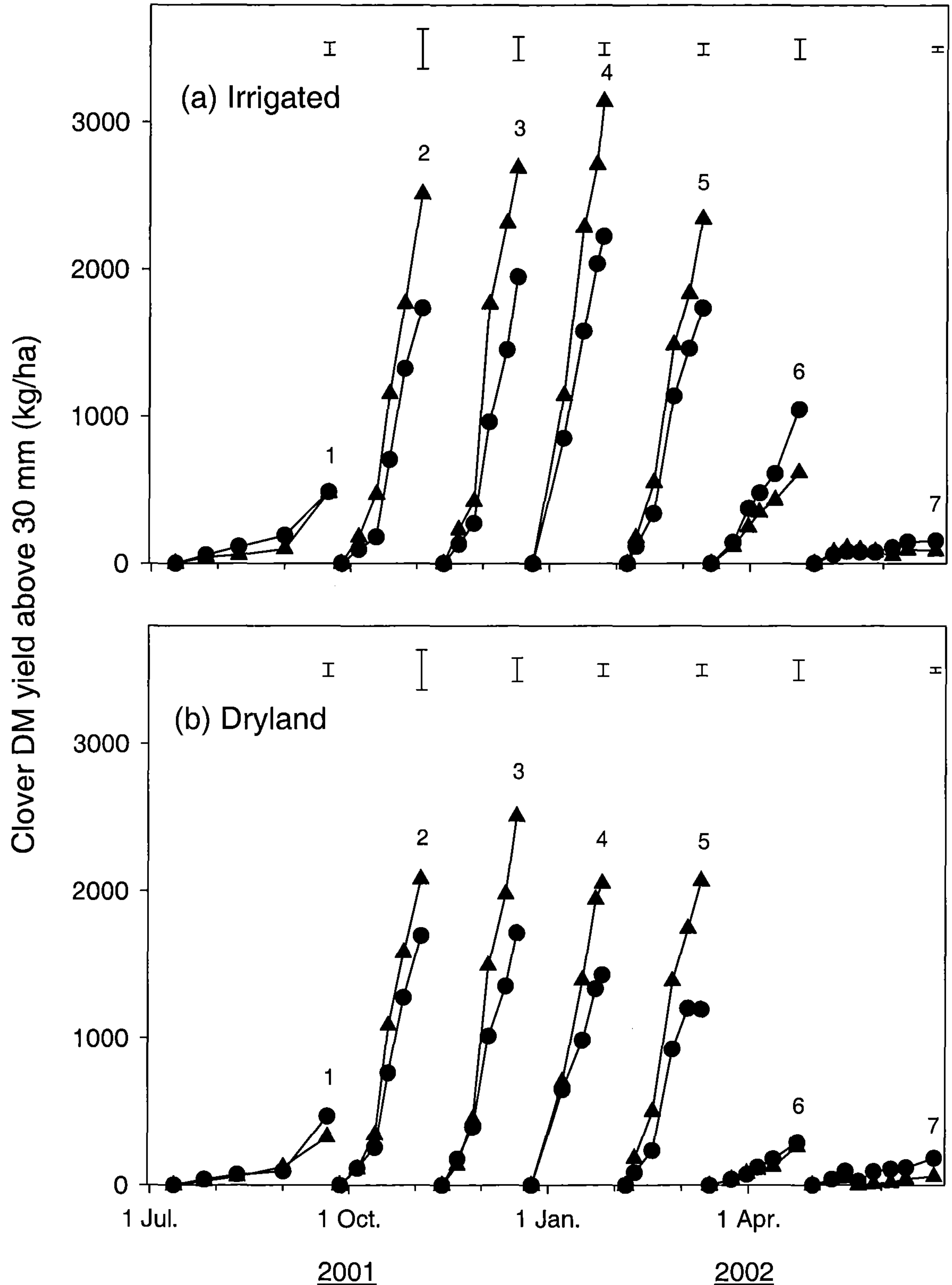

Figure 5.4 Clover dry matter (DM) yield accumulation (above 30 mm) for Caucasian (▲) and white (•) clovers under irrigated and dryland conditions from 1 July 2001 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean for final yields. Each regrowth period is numbered 1–7.

5.3.3.2 Clover dry matter production rates

Under irrigated conditions, production rates increased rapidly for Caucasian clover between July and mid-October and then increased at a slower rate to a mid-January peak of 98 kg DM/ha/d (Figure 5.5). This rate then declined to 16 kg DM/ha/d in March/April and 1 kg DM/ha/d in May/June. These rates were ~23 kg DM/ha/d higher (P<0.01) than those for white clover from mid-October to late February, but were ~10 kg DM/ha/d less (P<0.01) in March/April.

For both clovers, production rates were lower (P<0.05) under dryland than irrigated conditions from mid-January to March/April, but Caucasian clover growth was still ~23 kg DM/ha/d higher (P<0.01) than that for white clover in Periods 4–5 (Figure 5.5). Both clovers averaged 7 kg DM/ha/d in March/April without irrigation.

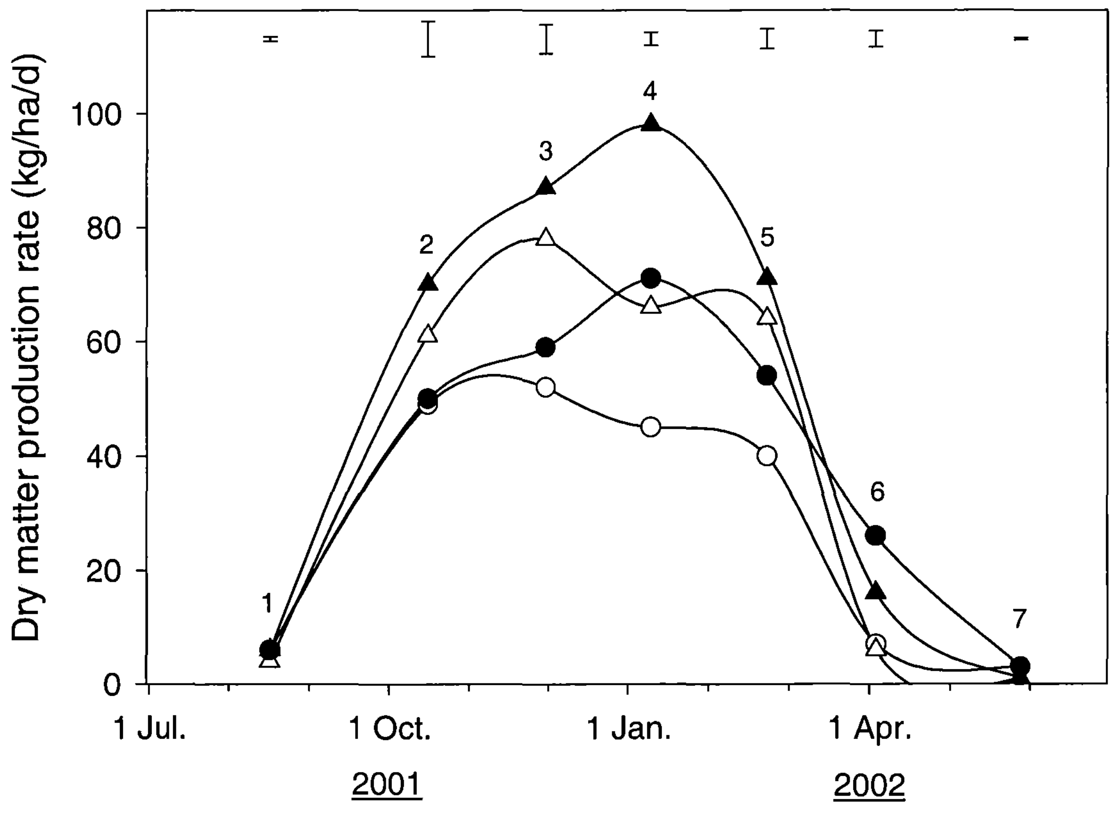

Figure 5.5 Dry matter production rate for Caucasian (▲,Δ) and white (•,o) clovers under irrigated (▲,•) and dryland (Δ,o) conditions from 1 July 2001 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean. Each regrowth period is numbered 1–7.

5.3.3.3 Relationship between clover dry matter production rate and temperature

The relationship between clover production rate and mean daily air temperature in the same regrowth period was examined under irrigated conditions (Figure 5.6). Production rates were linearly related ($R^2 \geq 0.97$; s.e. ≤ 6.3) to mean daily air temperature from 8 to 16 °C between July and January (Periods 1–4). This enabled the increase in spring/early summer growth to be estimated as 11 kg DM/ha/d for every degree increase in air temperature for Caucasian clover compared with 8 kg DM/ha/d for white clover.

However, between February and June (Periods 5–7) exponential functions ($R^2 \geq 0.98$; s.e. $\leq$ 6.1) were fitted to growth rates, which declined as air temperature dropped from 16 to 9 °C (Figure 5.6). It was notable that the production rate of Caucasian clover was affected more than that for white clover during this period. For example, Caucasian clover produced 16 kg DM/ha/d at 13 °C (Period 6) compared with 26 kg DM/ha/d for white clover. The DM production rate of white clover in autumn (Period 6) was about half that achieved for a similar temperature in spring (Period 2), but this difference was even greater for Caucasian clover.

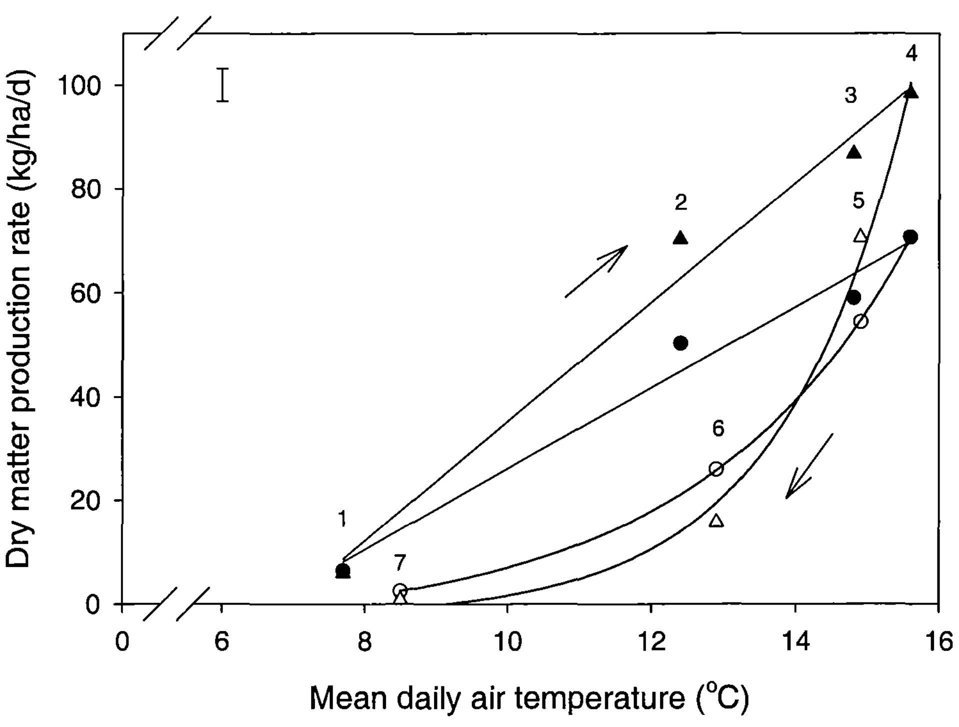

Figure 5.6 Relationship between dry matter production rate and mean daily air temperature for Caucasian (▲,Δ) and white (●,○) clovers under irrigated conditions from July–January (▲,●) and February–June (Δ,○) in 2001/02. Arrows indicate the direction of temperature change. The bar represents the maximum standard error for regressions. Each regrowth period is numbered 1–7.

5.3.4 Seasonal water use in 2001/02

Species comparisons of seasonal water use were concentrated on the 2001/02 season when both clover treatments had developed complete canopies. Data from the 2000/01 season are given in Appendix 12.

5.3.4.1 Water use

Seasonal water use was generally similar for both species regardless of irrigation treatment (Figure 5.7). For irrigated treatments, water use was ~1.4 mm/d in Period 1, and then increased to a maximum of 4.3 mm/d in January (Period 4) before declining to ~1.2 mm/d in May (Period 7). For dryland treatments, water use was ~0.7–1.3 mm/d less ($P<0.05$) than the irrigated treatments in November and January (Periods 3 and 4) and ~0.8 mm/d less in March/April (Period 6).

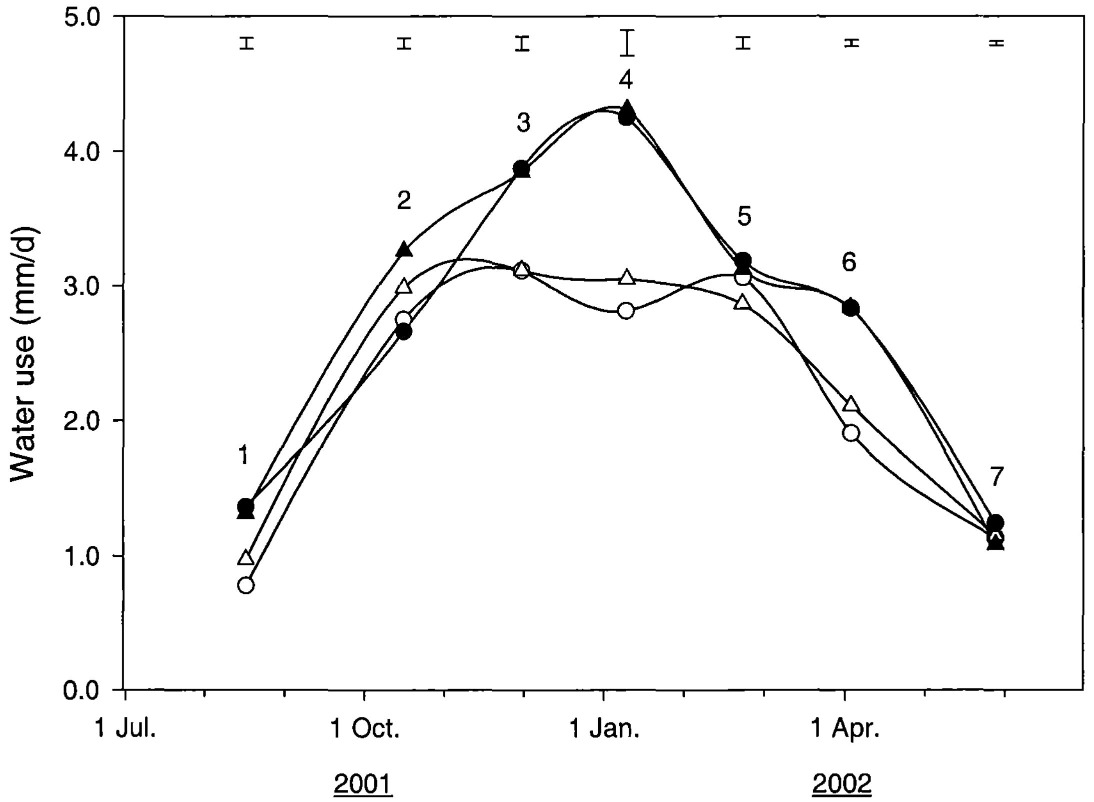

Figure 5.7 Seasonal water use for Caucasian (▲,Δ) and white (●,○) clovers under irrigated (▲,●) and dryland (Δ,○) conditions from 1 July 2001 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars indicate standard error of the mean. Each regrowth period is numbered 1–7.

5.3.4.2 Water use efficiency

Under irrigated conditions, WUE averaged 4.4 kg DM/mm for both species in Period 1 (Figure 5.8). WUE then increased for irrigated Caucasian clover to 16–19 kg DM/mm between Periods 2 to 5 before decreasing to 5 kg DM/mm in Period 6 and 1 kg DM/mm in Period 7. Caucasian clover WUE was ~4.0 kg DM/mm greater ($P<0.05$) than white clover WUE from Periods 3 to 5, but 3.0 kg DM/mm less ($P<0.05$) in Period 6.

There were no significant differences between irrigated and dryland Caucasian clover treatments in WUE from Periods 1 to 7 (Figure 5.8). Under dryland conditions, Caucasian clover WUE was 5.0–7.0 kg DM/mm greater ($P<0.05$) than white clover WUE from Periods 3 to 5, but was 2–3 kg DM/mm less ($P<0.05$) in Periods 1 and 7.

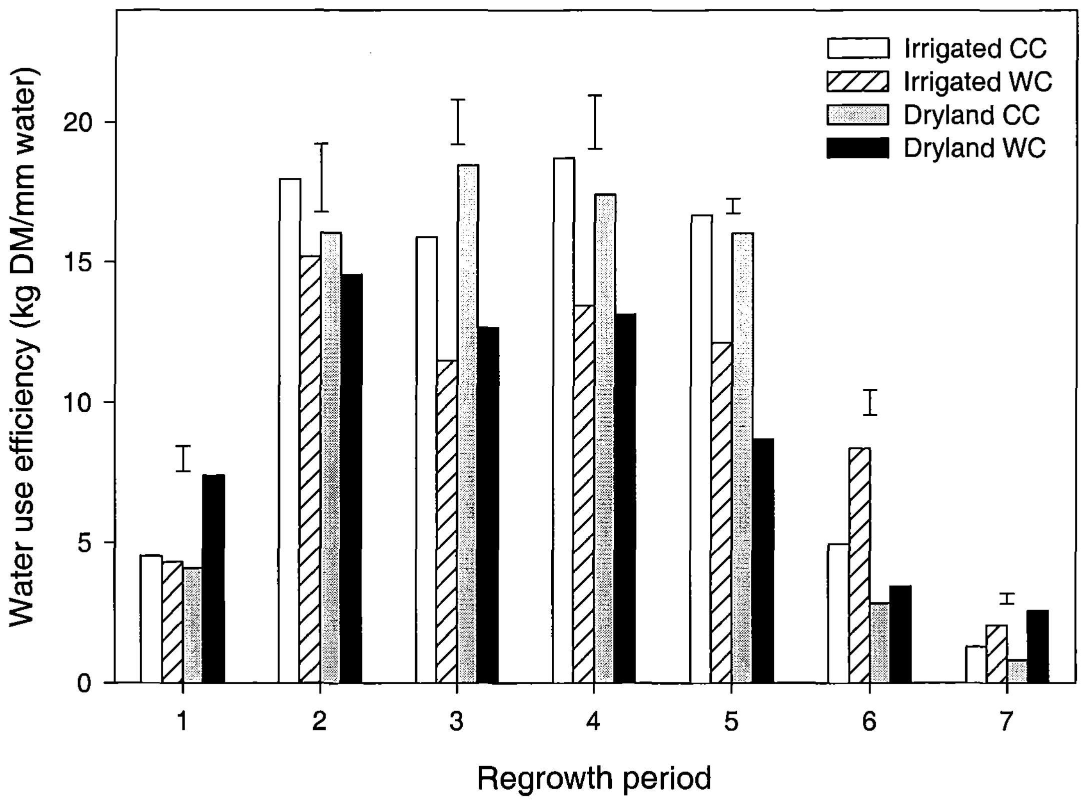

Figure 5.8 Seasonal water use efficiency for Caucasian (CC) and white (WC) clovers under irrigated and dryland conditions from 1 July 2001 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean.

5.3.5 Water extraction patterns in 2001/02

Species comparisons of water extraction patterns were concentrated on the 2001/02 season when both clover treatments had developed complete canopies. Data for the 2000/01 season are given in Appendix 13.

The water extraction patterns for the 3-year-old dryland clover pastures in 2001/02 are shown in Figure 5.9. The maximum extraction depth was estimated at ~1.15 m for both species, and both species extracted ~150 mm of total water above this depth. The water extraction patterns were similar between the two species for most of the rooting range (above 1.15 m) except that Caucasian clover extracted less water than white clover from 0.5 to 0.8 m depth.

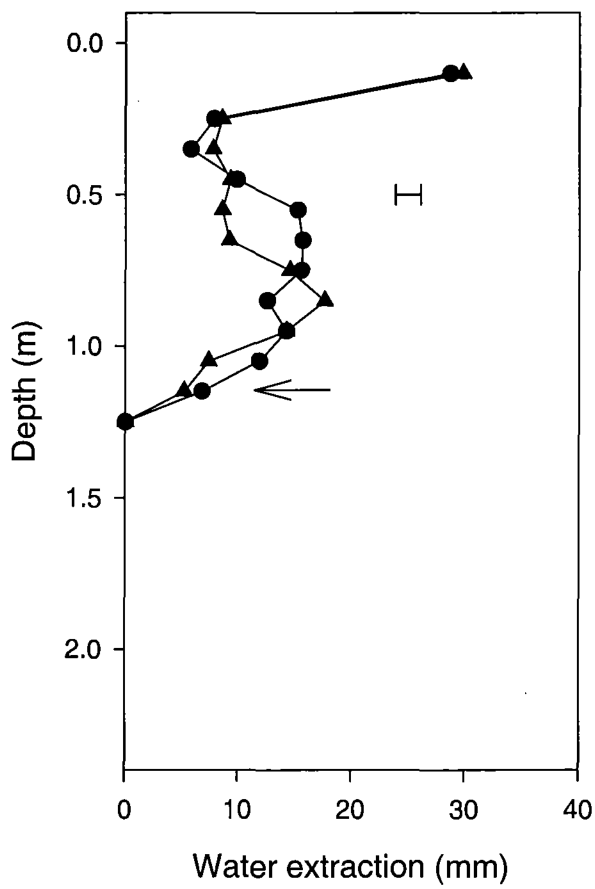

Figure 5.9 Water extraction patterns for 3-year-old Caucasian (▲) and white (•) clovers under dryland conditions measured from 1 Oct. 2001 to 22 Mar. 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bar represents the standard error of the mean. Arrow indicates the maximum water extraction depth.

5.3.6 Leaf photosynthesis rate

5.3.6.1 Relationship between leaf photosynthesis rate and temperature

Based on the fitted 'broken stick' model (R^2 = 0.94; s.e. = 1.8) the minimum leaf photosynthesis rate for Caucasian clover was 13 μmol $CO_2/m^2/s$ at the lowest measured air temperature of 7 °C (Figure 5.10). This rate then increased by an average of 1.5 μmol $CO_2/m^2/s$ per °C to a maximum of 37 μmol $CO_2/m^2/s$ at 23.7 °C before it declined by 2.3 μmol $CO_2/m^2/s$ per °C to be 27 μmol $CO_2/m^2/s$ at the highest measured air temperature of 28 °C. These rates were ~6 μmol $CO_2/m^2/s$ higher (P<0.05) than for white clover at all temperatures.

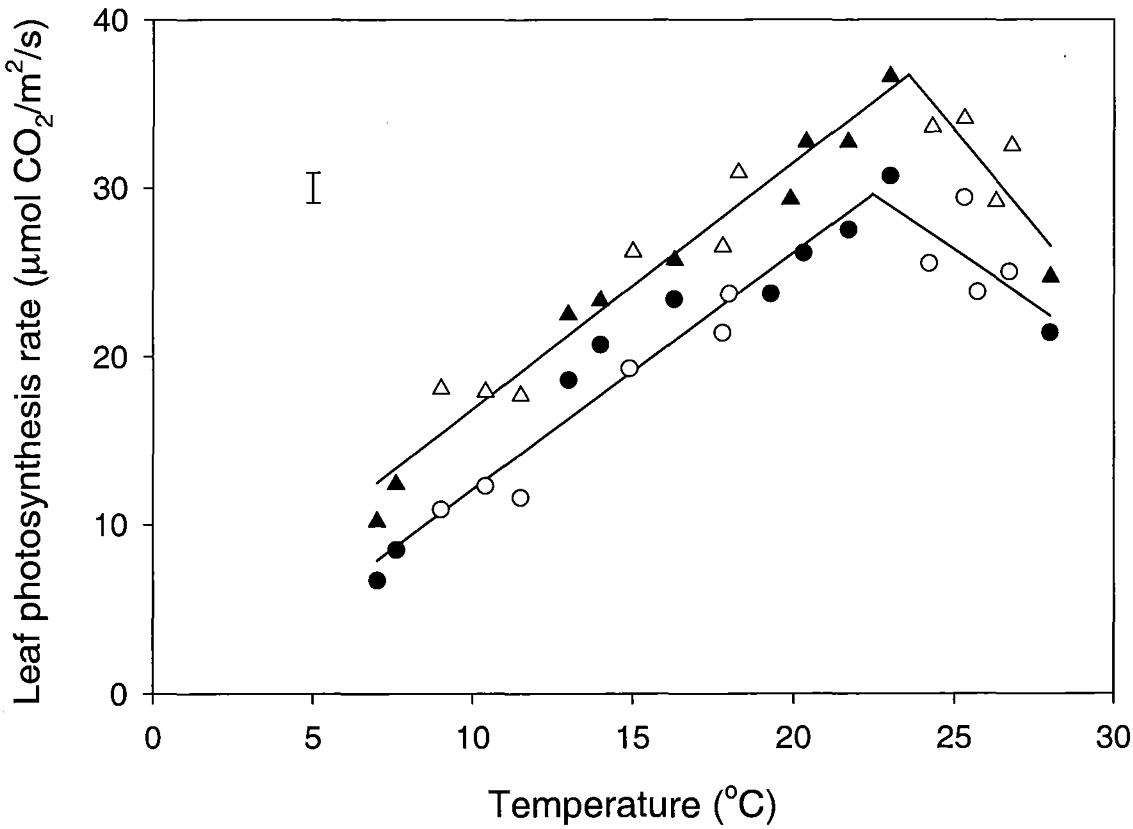

Figure 5.10 Response of net leaf photosynthesis rate for Caucasian (▲,Δ) and white (•,○) clovers to air temperature in non-limiting moisture (irrigated) conditions. Data are separated for periods 1 Jul.–31 Dec. (▲,•) and 1 Jan.–30 Jun. (Δ,○). Bar represents the standard error for the 'broken stick' model.

5.3.6.2 *Relationship between leaf photosynthesis rate and soil water content*

The relationship between leaf photosynthesis rate and soil water content was calculated when air temperature was in the optimum range of ~20–25 °C (Figure 5.10. Leaf photosynthesis rates were ~6 μmol $CO_2/m^2/s$ higher ($P<0.05$) for Caucasian than white clover as the ratio of TSWC:FC (to 1.7 m depth) decreased from 1.00–0.45 (Figure 5.11). For Caucasian clover, leaf photosynthesis rate was ~33 μmol $CO_2/m^2/s$ from 1.00–0.86 of FC, using the 'broken stick' model (R^2 = 0.84, s.e. = 2.7). This rate then declined by 3.9 μmol $CO_2/m^2/s$ per 0.10 unit change in TSWC:FC to a minimum of 17 μmol $CO_2/m^2/s$ at 0.45 of FC. White clover followed the same pattern, and leaf photosynthesis rates declined by 4.3 μmol $CO_2/m^2/s$ per 0.10 unit change in TSWC:FC to be 8 μmol $CO_2/m^2/s$ at 0.39 of FC.

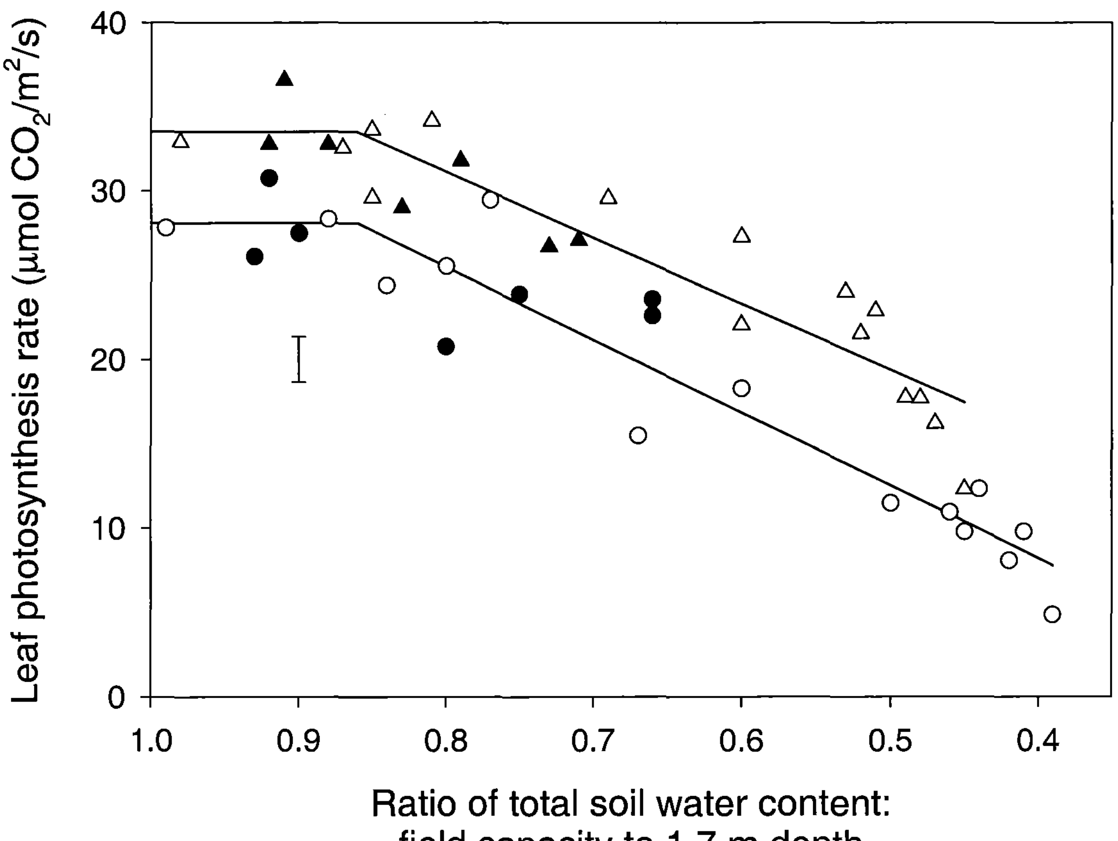

Figure 5.11 Response of net leaf photosynthesis rate for Caucasian (▲,Δ) and white (•,○) clovers to soil moisture – expressed as a ratio of total soil water content:field capacity (580 mm to 1.7 m depth) under non-limiting temperature (20–25 °C) conditions. Data are separated for periods 1 Jul.–31 Dec. (▲,•) and 1 Jan.–30 Jun. (Δ,○). Bars represent the standard error for the 'broken stick' model.

5.3.7 Leaf appearance rate

For white clover, leaf appearance rate was constant in Tt (T_b = 1 °C) in 2000/01 (Figure 5.12). This enabled the phyllochron (± s.e.) to be calculated as 126 ± 19.9 °Cd. For Caucasian clover, the phyllochron was 157 ± 25.9 °Cd from August to May (Periods 2–8), but increased to 314 ± 33.5 °Cd in July (Period 1) and 359 ± 42.7 °Cd in June (Period 9). These variable results indicate that T_b = 1 °C was incorrect for Caucasian clover. Thus, several T_b values were tested to minimise the coefficient of variation for the mean phyllochron, and a T_b of 5 °C was found to be most suitable. Using this higher T_b gave a similar phyllochron (126 ± 30.1 °Cd) to white clover. The phyllochron was ~14 °Cd longer for both species under dryland conditions (Appendix 14).

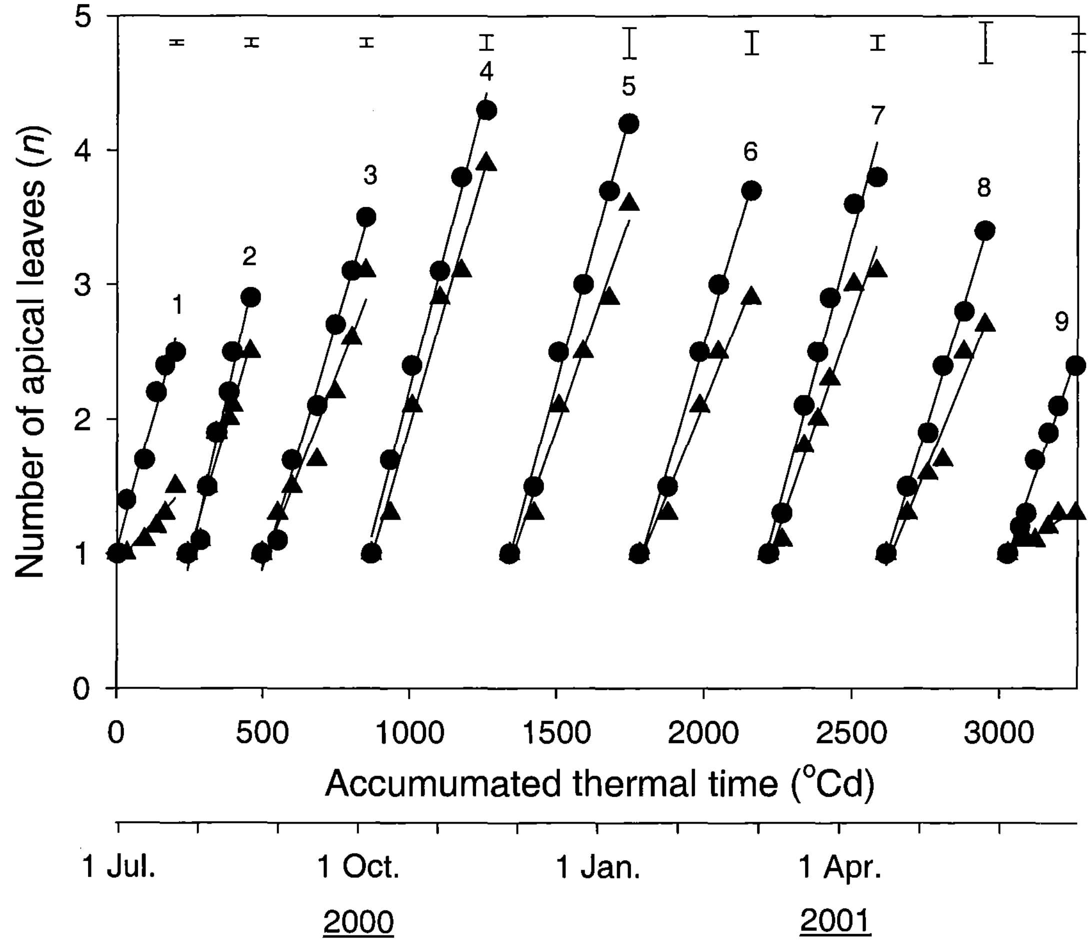

Figure 5.12 Leaf appearance rate for apical leaves of Caucasian (▲) and white (•) clovers as a function of accumulated thermal time under irrigated conditions from 1 Jul. 2000 to 30 Jun. 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean for final number of leaves. Each regrowth period is numbered 1–9.

5.4 Discussion

The establishment of monocultures of white and Caucasian clovers is uncommon in pastoral farming for a variety of practical reasons. However, this experiment has shown significant differences in WUE and in growth and development between the two species that can provide some explanation for their relative performance observed in mixed pastures.

5.4.1 Clover production in 2000/01

In this experiment only white clover had established fully by the second (2000/01) season. As a consequence, white clover produced ~6 t DM/ha more than Caucasian clover under irrigated conditions in that season (Table 5.3). This highlights the fact that Caucasian clover is slow to establish and supports the need for alternative strategies for establishment in pastures (Chapter 6).

Further analysis of the 2000/01 results was confounded by incomplete canopy closure and weed ingress (Table 5.3) in the irrigated Caucasian clover treatment and both dryland treatments, making species comparisons invalid. However, it was possible to use data from this season for measurements of photosynthesis and development which were based on individual leaves or plants and not the whole canopy.

5.4.2 Irrigated clover production in 2001/02

5.4.2.1 Annual yield

In their third year (2001/02), both clovers had established complete canopies, and the annual yield of Caucasian clover (11.9 t DM/ha) was 27% greater than that of white clover (9.4 t DM/ha) under irrigated conditions (Table 5.3). Caucasian clover yield was similar to an annual yield (~11.6 t DM/ha) reported for monocultures of 'Treeline' tetraploid Caucasian clover under similar irrigated and management conditions in Canterbury (Stewart and Daly, 1980). The yield of white clover monocultures was comparable to those previously reported (~10–13 t DM/ha) for white clover monocultures under irrigated conditions (Johns and Lazenby, 1973a; Vartha and Clifford, 1978).

5.4.2.2 *Seasonal production*

The greater annual production of Caucasian clover under irrigated conditions was due to greater yields than white clover from October to March (Figures 5.4a and 5.5). Temperature is the main environmental factor regulating solar radiation-driven plant growth under non-limiting moisture conditions (Section 2.6.1). The seasonal patterns of clover production offer insights into the timing and extent of temperature limitations operating on the two species.

5.4.2.2.1 Spring/early summer (July–January)

Clover yields were less than 0.6 t DM/ha from July to September (Figure 5.4a) when mean daily air temperatures were lowest (Figure 5.2). The relationships between clover production rate and mean daily air temperature showed that production rate increased linearly with increasing temperature from July to January (Figure 5.6). The greater yields of Caucasian clover in late spring/early summer (Figure 5.4) were due to a greater response (11 kg DM/ha/°C) than white clover (8 kg DM/ha/°C) to increasing mean daily air temperatures from 8 to 16 °C. This difference is consistent with previous observations of a greater response to increasing temperatures by Caucasian clover than white clover in rotationally grazed perennial ryegrass-based pastures during spring (Section 4.3.3).

The leaf photosynthesis results (Figure 5.10) provide an explanation for the difference in spring/summer production between the two species. It seems likely that the higher production rates for Caucasian clover in spring and summer were due to its higher leaf photosynthesis rate than white clover across the range of air temperatures experienced during this time. For both species, leaf photosynthesis rate increased with increasing temperature up to an optimum, which is consistent with the responses found for white clover (Woledge and Dennis, 1982) and cocksfoot (Peri *et al.*, 2002b) (Section 2.6.1.1).

Furthermore, in this experiment the optimum temperature range for photosynthesis of Caucasian and white clovers was the same. Specifically, the optimum temperature range to give >90% of the maximum leaf photosynthesis rate was approximately 21–25 °C. This range is consistent with the optimum temperature for white clover growth of 24 °C reported by Mitchell (1956) and Mitchell and Lucanus (1962). Thus, for any given canopy leaf area index, the canopy photosynthesis rate of Caucasian clover can be expected to exceed that of white clover and give more assimilate per unit of leaf area. Confirmation of this may explain the production advantage of Caucasian clover observed in perennial ryegrass-based pastures with irrigation (Section 4.3.1).

5.4.2.2.2 Late summer/autumn (January–June)

Clover production rates decreased exponentially with decreasing temperatures in late summer/autumn (Figure 5.6). However, both species had higher production rates in spring than at similar temperatures in autumn (e.g. Period 2 cf. Period 6). This result is consistent with different spring and autumn production rate responses to temperature observed for perennial ryegrass-based pastures in the previous experiment (Section 4.3.3). In the present experiment, soil water was non-limiting for clover production under irrigated conditions (Figure 5.3), which enabled temperature responses to be determined.

The leaf photosynthesis (Figure 5.10) and leaf appearance rate (Figure 5.12) results offer some insights into the reduced autumn production rates of Caucasian and white clovers. Firstly, the leaf photosynthesis rate responses to temperature for both species appeared to be the same irrespective of the time of year when measurements were taken (Figure 5.10). This indicates that the difference in spring and autumn production rate response to temperature was not caused by a change in the response of leaf photosynthesis rate, and suggests that other mechanisms were responsible.

One mechanism may be a change in the partitioning of DM. Specifically, the lower production rates in the autumn for both species (Figure 5.6) probably resulted from a greater partitioning of carbohydrates and protein to the roots and stolons/rhizomes to replenish reserves for over wintering and spring production. The storage of assimilates has been reported previously for white clover (Bouchart *et al.*, 1998) and Caucasian clover (Peterson *et al.*, 1994b). Furthermore, the greater reduction in production rate of Caucasian clover in the autumn (Period 6) indicated that it may have allocated a greater proportion of assimilate to rhizome and/or root storage than white clover.

It is also possible that a greater remobilisation of stored assimilates in spring contributed to the production advantage of Caucasian clover during this period. Peterson *et al.* (1994b) observed that total non-structural carbohydrate concentrations in crowns, rhizomes and roots of Caucasian clover increased in autumn and decreased in spring, indicating assimilate partitioning similar to that occurring in lucerne, which has a larger taproot than Caucasian clover (Brown, 2003; Moot *et al.*, 2003).

The slower leaf appearance rate of Caucasian clover in autumn (Figure 5.12) may have contributed to its lower production rate than white clover at this time. The phyllochron was the same for both species (126 °Cd). However, the higher T_b requirement for Caucasian clover (5 °C) than white clover (1 °C) used to calculate the phyllochron means that Caucasian clover took longer to accumulate the thermal time necessary to produce a new leaf. For example, assuming a mean air temperature of 10 °C, white clover is predicted to have produced a new leaf after 14 d compared with 25 d for Caucasian clover. This is because Caucasian clover would only accumulate 5 °Cd (heat units) each day due to its T_b of 5 °C compared with the T_b of 1 °C and an accumulation of 9 °Cd for white clover. The impact of a higher T_b will be greatest at the beginning and end of the growing season, when temperatures are most limiting. Thus, Caucasian clover can be expected to take longer to recover than white clover post grazing in autumn when air temperatures are declining.

Further research is necessary to determine if a change in the partitioning of carbohydrates and protein was the dominant factor responsible for the lower production of Caucasian clover in autumn, or if the higher base temperature was also a significant contributor. Understanding plant growth, development and assimilate partitioning has been important in making recommendations for optimum grazing management of lucerne (Moot *et al.*, 2003). In the present experiment, the growth and development results have provided a basis for understanding the seasonal production of Caucasian clover, and will also guide recommendations for its optimum grazing management in lowland pastoral systems. For example, the physiology of Caucasian clover suggests that a longer frequency between defoliations in autumn than in spring and summer may be required to assist Caucasian clover below ground root and rhizome production. This management would be expected to enhance above ground spring herbage production and result in prolonged persistence of Caucasian clover.

5.4.2.2.3 Winter

The higher T_b requirement for Caucasian clover also provides a physiological explanation for its perceived winter dormancy. Indeed, a "winter dormant" species can be simply quantified as one that has a higher T_b for development. This is consistent for other pasture species such as chicory, which is dormant in Canterbury for 3 months in winter and requires a T_b of 4.5 °C (Moot *et al.*, 2000). Species such as white clover with a base temperature of 1 °C, display greater cool season activity and normally have a longer growing season than those with a higher base temperature.

5.4.3 Dryland clover production in 2001/02

5.4.3.1 Annual production

Under dryland conditions, the annual yield of 3-year-old Caucasian clover (9.4 t DM/ha) was 34% greater than that of white clover (7.0 t DM/ha) in 2001/02 (Table 5.3). Caucasian clover yield was similar to the annual yield (~9.3 t DM/ha) reported for monocultures of tetraploid 'Treeline' Caucasian clover under dryland and similar management conditions in Canterbury (Stewart and Daly, 1980). The yield of white clover was similar to previously reported yields (6–7 t DM/ha) for dryland white clover monocultures (Johns and Lazenby, 1973b; Vartha and Clifford, 1978).

5.4.3.2 Seasonal production

The greater annual production for Caucasian clover was due to greater yields during periods of both non-limiting and limiting water conditions from October to March (Figures 5.4b and 5.5). The comparison of clover yields under irrigated and dryland conditions showed that soil water was limiting for clover production from January to June (Periods 4–7). At this time, the SWD was greater than 130 mm and the maximum SWD of 215 mm occurred in March (Figure 5.3).

5.4.3.2.1 Spring (July–December)

Dryland yields were similar to irrigated yields in the first three regrowth periods in spring, indicating that soil moisture was non-limiting for clover production (Figures 5.4b and 5.5). Caucasian clover yields were greater than those of white clover in the second and third regrowth periods. Thus, ~1.3 t DM/ha of Caucasian clover's annual production advantage under dryland conditions was due to greater spring production when soil water was adequate but low temperatures limited white clover production and leaf photosynthesis rates more than Caucasian clover (Section 5.4.2.2).

5.4.3.2.2 Summer (January–March)

Dryland yields were lower than irrigated yields from January onwards (Figure 5.4), indicating that soil water limited the production of both species during this time. However, Caucasian clover had greater yields (Figures 5.4b and 5.5) than white clover from January to March, which contributed to the other 1 t DM/ha of Caucasian clover's annual production advantage under dryland conditions. The difference was greatest in early March (Period 5) with Caucasian clover producing 0.8 t DM/ha more than white clover (Figure 5.4). This occurred when the SWD to 1.7 m depth was 186 mm (TSWC:FC ratio of 0.68), and when temperatures (Figure 5.2) were still adequate for the growth of both species (21–25 °C, Figure 5.10).

The leaf photosynthesis results (Figure 5.11) provide an explanation for the greater production of Caucasian clover than white clover under dryland conditions. For both species, the optimum soil moisture range to give >90% of the maximum leaf photosynthesis rate was the same at 1.00–0.86 of FC. The onset of moisture stress resulted in a similar decrease in leaf photosynthesis rate, but the leaf photosynthesis rate for Caucasian clover was higher than for white clover irrespective of soil moisture. These responses to increased water stress are consistent with responses found for white clover (Johns, 1978) and cocksfoot (Peri *et al.*, 2002b) (Section 2.6.2.1). Thus, for any given canopy leaf area index, the canopy photosynthesis rate of Caucasian clover can be expected to exceed that of white clover and give more assimilate per unit of leaf area. Confirmation of this may explain the production advantage of Caucasian clover over white clover observed in perennial ryegrass-based pastures under summer dry conditions (Black and Lucas, 2000; Watson *et al.*, 1998).

5.4.4 Water use efficiency in 2001/02

In their third year (2001/02), Caucasian clover produced ~2.5 t/ha more DM than white clover under both irrigated and dryland conditions, but the quantity of water used was similar for both species (Table 5.3). Therefore, Caucasian clover produced more DM for the same amount of water used than white clover, indicating greater WUE. This result was particularly apparent during spring and summer (Periods 3 to 5) when Caucasian clover production rates were ~23 kg DM/ha/d greater than those of white clover (Figure 5.5), but the rate of water use was the same (Figure 5.7). The lower WUE of white clover during this period (Figure 5.8) is consistent with the commonly accepted view that this species is inefficient in controlling water loss (Aparicio-Tejo *et al.*, 1980a; Burch and Johns, 1978; Johns, 1978; Johns and Lazenby, 1973b). In contrast, the greater WUE of Caucasian clover under dryland conditions provides an explanation for its greater productivity than white clover in this environment (Black and Lucas, 2000; Watson *et al.*, 1998).

It seems likely that the greater leaf photosynthesis rates of Caucasian clover over white clover at any given soil water content (Figure 5.11) contributed to the greater WUE by Caucasian clover. A more accurate explanation of this result would require more detailed analysis of factors such as the rate of transpiration of leaves and canopy development. However, there are a number of possible explanations for this difference. Firstly, the leaf stomatal resistance may be greater for Caucasian than white clover in response to water stress, giving Caucasian clover more control of transpiration water loss. This has been found for other drought tolerant pasture species such as tall fescue and phalaris (Johns, 1978). Secondly, the most immediate response to water stress is decreased leaf size, which would affect canopy leaf area, light interception and therefore yield (Burch and Johns, 1978; Karsten and MacAdam, 2001). Any differences in these responses between Caucasian and white clovers would be related to their relative ability to control transpiration loss, or access to greater soil water supply via deeper root systems.

5.4.5 Water extraction patterns

It remains to be seen if the taproot of Caucasian clover is able to extract water from greater depths than white clover and therefore provide an additional advantage from access to more water during summer. Indeed, the current lack of any differences in water extraction depth (Figure 5.9) suggests that white clover still had an active taproot in its third year. However, it has long been recognised that white clover looses its seedling taproot within 1–2 years, after which clonal white clover plants are dependent on their shallow nodal root systems (Brock *et al.*, 2000; Westbrooks and Tesar, 1955).

Evans (1978) found that a mature white clover monoculture had most of its roots in the top 0.20 m of soil, but could extract water to a depth of 0.90 m, which is ~0.20 m less than the extraction depth found in the present study. In contrast, lucerne extracted water from at least 2.10 m due to its deep taproot. It is also possible that some white clover plants may have established from reseeding in 2000/01, which means that their taproots might still have been present during 2001/02. Any potential advantage from greater rooting depth for Caucasian clover would not be expected until after the tap root of white clover has senesced.

5.5 Conclusions

This experiment has quantified reasons for differences in seasonal DM production observed between Caucasian and white clovers. The results confirm that Caucasian clover can increase clover production in environments where high quality pastures are required in spring and summer. Caucasian clover may not be suited to environments and farming systems where cool season production is required, but it is suited to cool temperate environments with warm moist summers where persistence is important. Specific conclusions were:

1. In its third year (2001/02), when complete canopy closure was achieved, Caucasian clover produced ~2.5 t DM/ha more annual yield than white clover under irrigated conditions due to 20–25 kg DM/ha/d higher production rates in spring and summer, when mean daily air temperature was increasing from 8 to 16 °C.
2. The production advantage for Caucasian clover in spring and summer moist conditions occurred when its leaf photosynthesis rate was ~6 μmol $CO_2/m^2/s$ higher than white clover. Both clovers had a similar range of optimum temperature (21–25 °C) for photosynthesis.
3. The yield advantage of Caucasian clover was maintained under dryland conditions due to 20–25 kg DM/ha/d higher production rates in summer, when soil water deficit was below 130 mm.
4. The production advantage for Caucasian clover in summer dryland conditions occurred when its leaf photosynthesis rate was ~6 μmol $CO_2/m^2/s$ higher than white clover irrespective of soil moisture. Both clovers had a similar range of optimum soil moisture (1.00–0.86 of field capacity) for photosynthesis.
5. Caucasian clover extracted water from similar depths (up to 1.15 m) as white clover, but Caucasian clover had a 5.0–7.0 kg DM/mm higher water use efficiency that contributed to its greater production under summer dryland conditions.
6. Caucasian clover had a phyllochron of 126 ± 30.1 °Cd, which was the same as white clover, but the T_b requirement was higher for Caucasian (5 °C) than for white clover (1 °C).

Chapter 6

Sowing strategies for successful establishment of Caucasian clover and white clover

6.1 Introduction

In this thesis, results have shown that Caucasian clover is more productive than white clover in irrigated ryegrass pastures under both high (Olsen P 20 μg/ml, sulphate-S 12 μg/g) and low (Olsen P 11 μg/ml, sulphate-S 7 μg/g) soil fertility conditions (Chapters 3 and 4). Also, Caucasian clover has shown potential as a dryland legume with greater spring and summer production than white clover (Chapter 5). Despite these positive results, slow establishment of Caucasian clover is a major reason why farmers in New Zealand are reluctant to adopt this species for their permanent pastures.

The sowing strategies required for successful establishment of Caucasian clover in tussock grasslands have been emphasised in Section 2.5 (Lowther *et al.*, 1998; Moorhead *et al.*, 1994). However, modified strategies are required for successful establishment of Caucasian clover in more intensive lowland pastures (Hurst *et al.*, 2000; Widdup *et al.*, 1998). Under these conditions, sowing time and ryegrass seeding rate are important factors influencing the success of slow establishing pasture species (Dumbleton, 1997; Moot *et al.*, 2000). The majority (83%) of New Zealand pastures are autumn sown (Sangakkara *et al.*, 1982) with commercial recommendations of 16–20 kg/ha of perennial ryegrass (Charlton, 1991). This contradicts the scientific literature that shows most slow establishing perennial pasture species benefit from spring sowing and ryegrass seeding rates of less than 10 kg/ha (Cullen, 1958; Dumbleton, 1997). The reduction in ryegrass seeding rate reduces inter-plant competition for limited resources, particularly light (Brougham, 1953), with minimum impact on total yield (Culleton, 1986; Dumbleton, 1997).

Thus, the objective of the research described in this chapter was to evaluate the impact of sowing time and perennial ryegrass seeding rate on the establishment of Caucasian and white clovers in permanent pastures. In this study, an acceptable pasture was defined as one that had a high (>15%) clover content and suppressed weeds (<5%) with minimum impact on total yield in the first spring after sowing.

6.2 Materials and methods

6.2.1 Site description

The experimental area was located in Iversen Field block 10, adjacent to the field experiment described in Chapter 5. Section 5.2.1 described the experimental site and meteorological conditions.

6.2.2 Seeding and design

The experiment used a split-plot factorial randomised complete block design. Main plots were four sowing dates of 24 September 1999 (SD1), 9 November 1999 (SD2), 4 February 2000 (SD3) and 31 March 2000 (SD4). Sub-plots of 'Grasslands Demand' white clover (2 kg/ha) and 'Endura' Caucasian clover (8 kg/ha) were sown with 0, 3, 6 or 12 kg/ha of 'Nui' perennial ryegrass infected with the AR1 strain of *Neotyphodium lolii.* There were two replicates giving a total of 64sub-plots. Sub-plots were 2.1 x 6.0 m drilled with an Øyjoord cone seeder at 150 mm row spacing and a target depth of 15 mm. Clover seed was lime-coated and inoculated with the *Rhizobium trifolii* strain CC275e for white clover and ICC148 for Caucasian clover.

Before SD1, the experimental area was cultivated using conventional methods to produce a firm and fine textured seedbed. Sulphur superphosphate (8% P, 19% S) at 250 kg/ha was applied during cultivation, based on soil test results from Iversen 10 in July 1999 (Table 5.1). Before the second and subsequent sowings, the non-residual herbicide 'Buster' (a.i. glufosinate-ammonium; 600 g a.i./ha) was sprayed to kill any emerged weeds.

6.2.3 Management

Pastures were first defoliated when individual Caucasian clover plants had four–five trifoliate leaves. A rotary lawn mower was used for the first defoliation of SD1 pastures, but grazing by young sheep was used for the other three sowing dates. First defoliations were SD1: 64 days after sowing (DAS) on 27 November, SD2: 12 January (64 DAS), SD3: 2 April (57 DAS) and SD4: 30 June (91 DAS). Pastures were subsequently grazed at 5–8 week intervals when 1500–2500 kg DM/ha had accumulated in the pastures that included perennial ryegrass.

The herbicide '2,4-DB' (a.i. 2,4-DB) at 2400 g a.i./ha was sprayed on 10 November 1999 and 23 December 1999 to control mainly wild turnip (*Brassica rapa* spp.) and wireweed which were prevalent in SD1 pastures. The insecticide 'Chlor-P 480EC' (a.i. chlorpyrifos) at 240 g a.i./ha was sprayed on 24 November 1999 to control greasy cutworm that caused ~20% loss of emerged clover plants in SD2.

Plots received 120 mm of irrigation water from December 1999 to March 2000, based on a soil water budget (Equation 5.1) to prevent a deficit of 25 mm to 0.50 m soil depth.

6.2.4 Measurements

6.2.4.1 Soil and air temperatures

Directly after sowing, soil temperature sensors (Thermistors KTY-110) were placed in four sub-plots at a depth of 15 mm. One sensor was placed in each of the four sowing date treatments. Air temperature was recorded from a single sensor placed in a Stevenson screen on site. Temperatures were recorded every 5 minutes and integrated and logged every hour with a data logger (DT100 data-taker) to determine daily mean temperatures (Figure 6.1).

6.2.4.2 Dry matter production and botanical composition

DM production was determined from pre-grazing herbage cuts to 30 ± 5 mm above ground from one randomly placed 0.2 m^2 quadrat in each sub-plot. Sub-samples were taken to determine botanical composition before dry weight was measured. The fresh sub-samples, containing 200–400 pieces, were separated into white clover, Caucasian clover, perennial ryegrass, and weeds, and dried separately to determine the contribution of each species to total yield. Some volunteer white clover (presumably 'Huia') was detected in the Caucasian clover treatments, and this was included in the weed component.

6.2.4.3 Nitrogen % of ryegrass herbage

Dried sub-samples of ryegrass from harvests on 12 January 2000, 30 June 2000 and 6 November 2000 were retained for determination of N % using the Kjeldahl procedure. Ryegrass samples from the two replicates of selected treatments (24 September and 31 March sowing dates with 3 and 12 kg/ha of perennial ryegrass) were bulked and ground in a mill to pass through a 1-mm stainless steel sieve. Analyses were performed by the Animal and Food Sciences Division, Lincoln University, Canterbury, New Zealand.

6.2.4.4 Seedling growth and development

After each sowing, the middle metre of two adjacent non-border rows was marked in each sub-plot. The number of emerged seedlings was recorded daily until seedlings ceased to emerge. Emergence was considered to have occurred when both cotyledons of the clover species had expanded and when the coleoptile of perennial ryegrass was visible (Moot *et al.*, 2000). The number of days to reach 50% of the final emergence was determined by interpolation.

Plant population was determined at the time of first defoliation from plant counts within two randomly placed 0.2 m^2 quadrats in each sub-plot. Also at this time, 10 plants per sub-plot were dug to a 0.25 m depth from two adjacent non-border rows of each sub-plot. The plants were washed and separated into shoots and roots. Shoots included leaves, petioles (clovers) and branch (stolons or crown shoots) or tiller structures. The number of leaves and branch or tiller structures were recorded before the dry weight (DW) of shoots and roots was measured.

6.2.5 Statistical analysis

Significant ($P<0.05$) treatment effects were determined by ANOVA procedures (GenStat, 1997). Data from each harvest were analysed according to the full split-plot factorial design. The exception was the first harvest of SD1 which was analysed as a two-way factorial of clover species × ryegrass seeding rate. Also, separate two-way analyses were performed on final botanical composition and DM production data from each sowing date. Botanical composition percentages were arc-sine transformed as necessary using the method described in Section 3.2.11. Standard errors of the means (s.e.m.) are reported for each measured variable. Treatment means were separated by Fisher's Protected l.s.d. test ($\alpha=0.05$) whenever the ANOVA indicated that differences among treatments were significant.

The time to 50% emergence for each sowing date was analysed separately, with maximum standard errors (s.e.) reported. The thermal time (Tt, in °Cd) to 50% emergence was determined using daily maximum and minimum 15-mm soil temperature data (Figure 6.1) in Equation 2.5. The T_b was assumed to be 0 °C for all three species (Moot *et al.*, 2000).

6.3 Results

6.3.1 Soil and air temperatures

The mean 15-mm soil temperature increased from ~13 to 17 °C between the 24 September and 4 February sowing dates, but then decreased rapidly to ~11 °C for the 31 March sowing date and 5 °C in late June before increasing again in spring (Figure 6.1). There were differences in the mean air and soil temperatures, particularly in late spring and early summer when the soil temperatures were 2–3 °C warmer than screen temperatures.

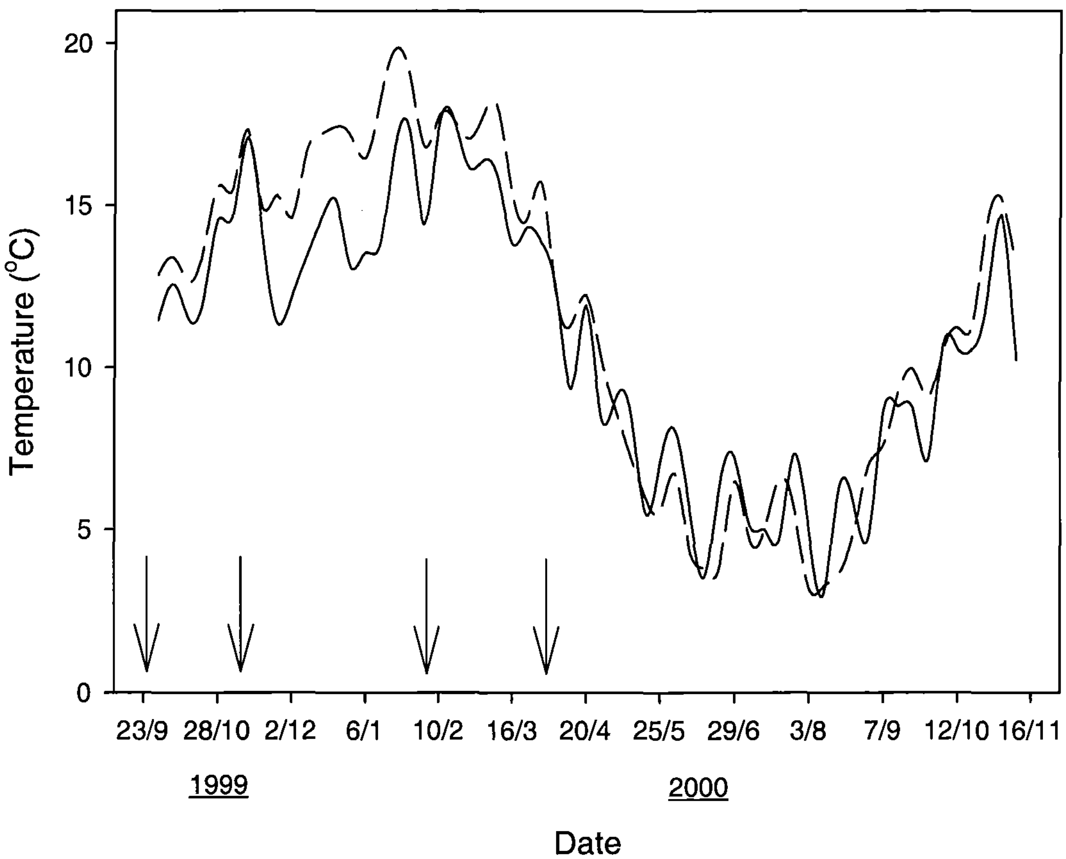

Figure 6.1 Mean 7-day screen (——) and 15-mm soil (----) temperatures for block 10 Iversen Field at Lincoln University, Canterbury, New Zealand. Arrows indicate sowing dates.

6.3.2 Botanical composition

6.3.2.1 Spring sowing dates

On 6 November 2000, the botanical composition of the 14-month-old pastures sown on SD1 showed similar clover and weed contents to the 12-month-old pastures sown on SD2 (Figure 6.2). Clover and weed contents were highest in monocultures of each clover species, but decreased exponentially as ryegrass seeding rate increased. For SD1, the addition of ryegrass reduced white clover content from 91% in monocultures to about 28% with 3 to 12 kg/ha of ryegrass, but weed content was always ≤1% when ryegrass was included. In comparison, SD1 Caucasian clover pastures had 47% sown clover and 53% weeds in the monoculture and ryegrass reduced the sown clover content to about 7% at all seeding rates. For SD2, white clover content was reduced from 24% at 3 kg/ha to 15% at 12 kg/ha compared with 8% to 1% for Caucasian clover, but the weed content was always ≤2% with ryegrass. The predominant weeds during spring were hawksbeard (*Crepis capillaris*), wireweed and annual poa.

6.3.2.2 Autumn sowing dates

On 6 November 2000, the 9-month-old pastures sown on SD3 had more (P<0.001) clover and fewer weeds than the 7-month-old pastures sown on SD4 regardless of ryegrass seeding rate (Figure 6.3). Clover and weed contents were highest in monocultures of each clover species, but decreased exponentially as ryegrass seeding rate increased. For SD3, the addition of ryegrass reduced the white clover content from 26% at 3 kg/ha to 13% at 12 kg/ha, but there were no weeds when ryegrass was included. In contrast, SD3 Caucasian clover pastures had 66% weeds (hawksbeard, wireweed, annual poa, volunteer white clover) and only 34% sown clover in the monoculture and ≤1% sown clover when ryegrass was included. For SD4, neither clover was more than 8% of the composition when ryegrass was included and the weed content was 2–15%. The predominant weeds were the winter annuals chickweed and annual poa.

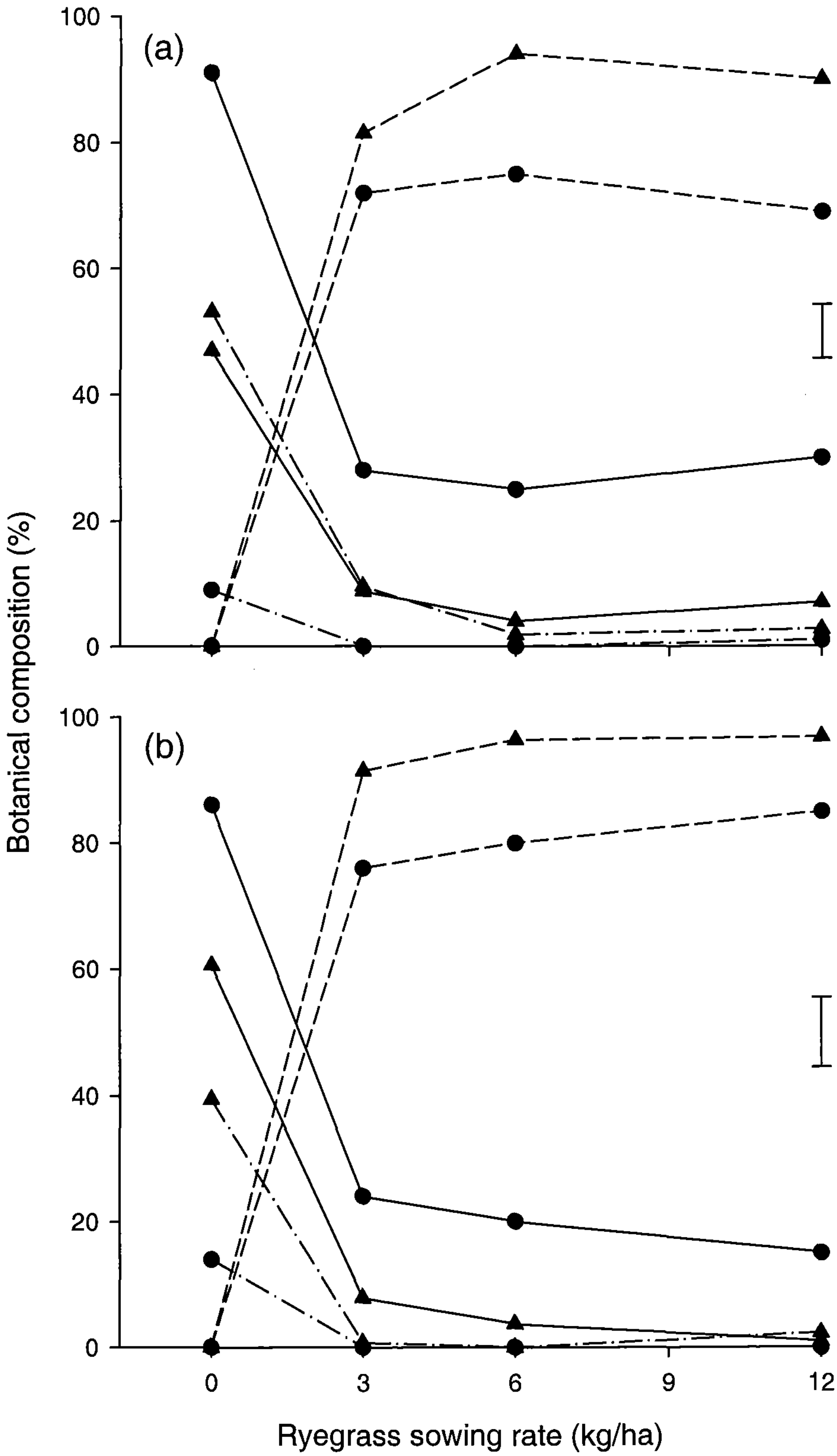

Figure 6.2 Percentage of ryegrass (----), clover (——) and weeds (–·–·) on 6 November 2000 in Caucasian (▲) and white (•) clover pastures sown on (a) 24 September 1999 and (b) 9 November 1999 with four perennial ryegrass seeding rates. Bars represent the maximum standard error of means.

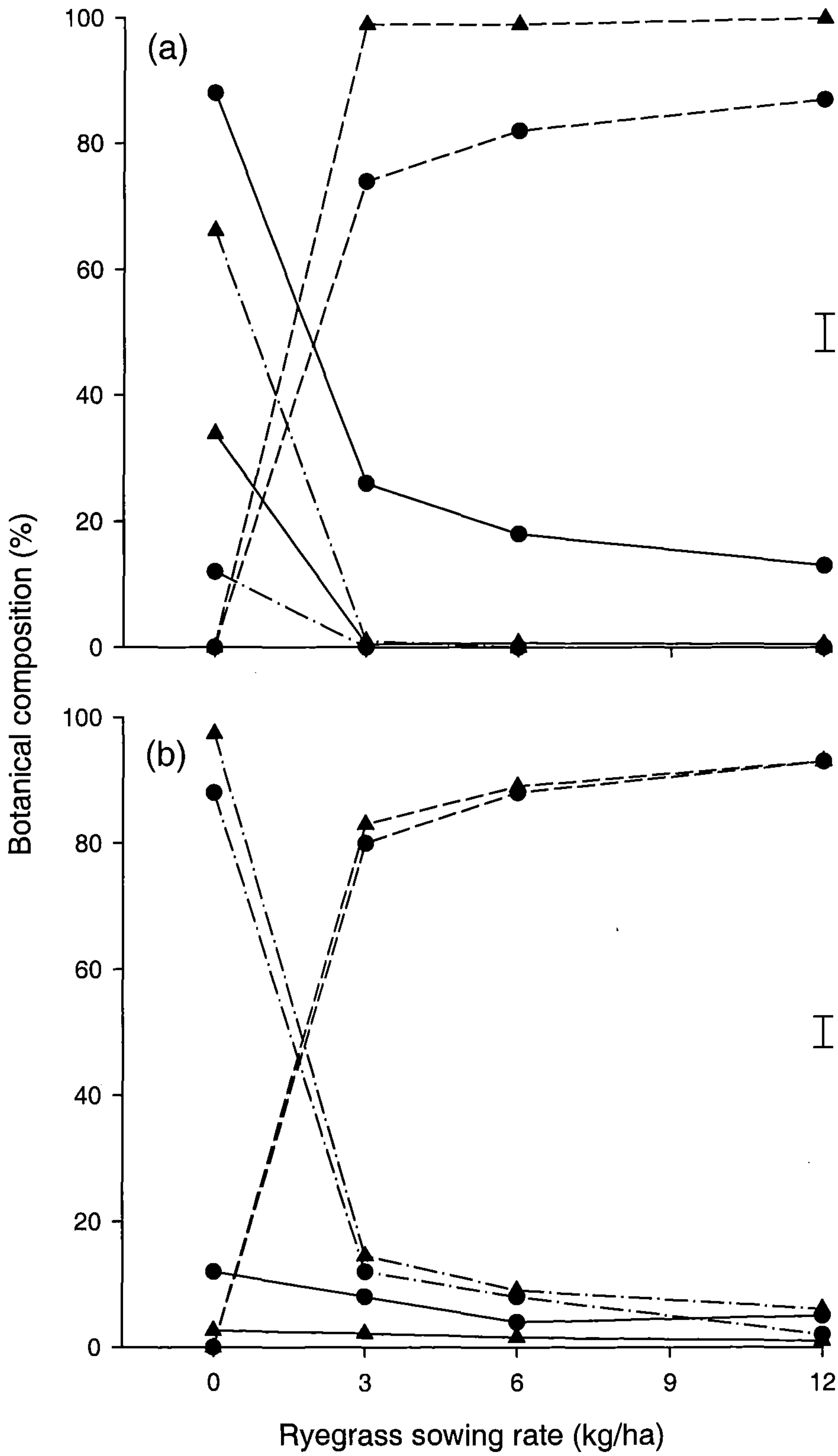

Figure 6.3 Percentage of ryegrass (----), clover (——) and weeds (–·–) on 6 November 2000 in Caucasian (▲) and white (•) clover pastures sown on (a) 24 September 1999 and (b) 9 November 1999 with four perennial ryegrass seeding rates. Bars represent the maximum standard error of means.

6.3.3 Total dry matter production

Total DM production up to 6 November 2000 was affected (*P*<0.05) by the interactions of sowing date × ryegrass seeding rate, sowing date × species and ryegrass seeding rate × species. However, the small 'F' ratios (<9.55) for these interactions compared with species (29.86), ryegrass seeding rate (249.24) and sowing date (273.14) main effects meant that they were comparatively unimportant. Therefore, this section focuses on the clover species and ryegrass seeding rate main effects analysed within each sowing date.

6.3.3.1 Spring sowing dates

For SD1, total DM production up to 6 November 2000 was greater (*P*<0.01) from white clover than Caucasian clover pastures, and increased (*P*<0.001) from 9990 kg/ha in clover monocultures to 16 350 kg/ha with 6 and 12 kg/ha of ryegrass (Table 6.1). Similarly, for SD2 total DM was greater (*P*<0.05) for white clover than Caucasian clover, and clovers averaged 6870 kg DM/ha as monocultures and 14 510 kg DM/ha with 6 and 12 kg/ha of ryegrass.

6.3.3.2 Autumn sowing dates

For SD3, total DM production up to 6 November 2000 was greater (*P*<0.05) for white clover than Caucasian clover pastures, and increased (*P*<0.001) from 5390 kg/ha in clover monocultures to a maximum of 9150 kg/ha with 6 kg/ha of ryegrass (Table 6.1). For SD4, total DM production was 2620 kg/ha without ryegrass but increased with increasing ryegrass seeding rates up to 6760 kg/ha at 12 kg/ha, regardless of clover species.

Table 6.1 Total dry matter production up to 6 November 2000 from Caucasian (CC) and white (WC) clover pastures sown on four dates with different ryegrass seeding rates.

	Sowing date			
	24 Sep. '99	9 Nov. '99	4 Feb. '00	31 Mar. '00
	kg DM/ha			
Clover (C)				
CC	13 620$_b$	11 690$_b$	7310$_b$	5400
WC	15 150$_a$	12 710$_a$	8110$_a$	5550
s.e.m.	241	300	231	76
P_C	0.003	0.046	0.043	0.202
Seeding rate (R)				
0 kg/ha	9990$_c$	6870$_c$	5390$_c$	2620$_c$
3	14 840$_b$	12 900$_b$	7820$_b$	6240$_b$
6	16 410$_a$	14 220$_{ab}$	9150$_a$	6300$_b$
12	16 290$_a$	14 810$_a$	8480$_{ab}$	6760$_a$
s.e.m.	340	424	327	107
P_R	<0.001	<0.001	<0.001	<0.001

Note: Within columns and main effects, values with the same or no letter subscript are not significantly different (α=0.05).

6.3.4 Seedling growth and development

6.3.4.1 Emergence

The number of days to 50% seedling emergence was similar for all three species, but ranged from ~8 d from SD3 to ~13 d for SD4 (Table 6.2). Based on 15-mm soil temperatures, the Tt requirement to 50% emergence was unaffected by sowing date and similar at ~146 °Cd (T_b = 0 °C) for all three species.

Table 6.2 Number of days and thermal time to reach 50% of final emergence for Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) sown on four dates. Data presented are averaged over all ryegrass seeding rates.

	Sowing date			
	24 Sep. '99	9 Nov. '99	4 Feb. '00	31 Mar. '00
Days to 50% emergence				
CC	11.4	9.1	7.9	13.0
WC	11.8	9.0	8.0	11.4
PR	11.6	9.8	8.0	12.2
Maximum s.e.	1.83	1.40	0.93	1.52
Thermal time (°Cd) to 50% emergence				
CC	158	146	146	158
WC	162	144	148	138
PR	161	157	148	148
Maximum s.e.	22.1	21.5	17.8	18.2

Note: n = 8 for clovers and n = 12 for perennial ryegrass.

6.3.4.2 Plant population

Examination of individual seedlings at the first defoliation of each sowing date showed white clover averaged 60 plants/m^2 compared with 51 plants/m^2 (P<0.01) for Caucasian clover (Table 6.3). Clover plant population averaged 75 plants/m^2 for SD1 and SD3 compared with 37 plants/m^2 (P<0.01) for SD2 and SD4, and decreased from 62 to 46 plants/m^2 as ryegrass seeding rate increased from 0 to 12 kg/ha. Ryegrass plant population averaged 132 plants/m^2 for SD2–4 compared with 106 plants/m^2 (P<0.05) for SD1, and increased (P<0.001) from 79 to 275 plants/m^2 with increasing seeding rates.

6.3.4.3 Shoot and root dry weight

Shoot DW of clovers sown on SD1–3 averaged 48 mg/plant compared with 14 mg/plant (*P*<0.05) when sown on SD4 (Table 6.3). Root DW of clovers sown on SD1–3 averaged 22 mg/plant compared with 10 mg/plant (*P*<0.05) when sown on SD4. For ryegrass, shoot DW averaged 950 mg/plant from SD2 and 727 mg/plant from SD1 and SD3, but this was halved for SD4 (*P*<0.001). Root DW of ryegrass averaged 177 mg/plant from SD1–3, but this was halved for SD4 (*P*<0.05). Average shoot DW of clover plants decreased (*P*<0.05) from 43 to 36 mg/plant as ryegrass seeding rate increased from 3 to 12 kg/ha. Average root DW of clovers decreased (*P*<0.05) from 22 to 17 mg/plant as ryegrass seeding rate increased from 3 to 12 kg/ha.

6.3.4.4 Number of leaves and branches/plant

The number of leaves per clover plant was influenced (*P*<0.001) by the interaction of clover species × sowing date (Table 6.3). Specifically, the number of leaves on white clover decreased from 9.4 to 4.8 between SD2 and SD4, but only from 4.8 to 3.4 for Caucasian clover (Table 6.4). Individual Caucasian clover plants had no crown shoots (branches) at first defoliation compared with an average of one stolon initial on individual white clover plants. The number of stolons/plant for white clover decreased with increasing ryegrass seeding rate and delayed sowing date but the differences were not significant.

For ryegrass, the average number of leaves/plant was about 16.0 when sown on SD1–3, but only 9.8 (*P*<0.01) when sown on SD4 (Table 6.3). Similarly, the average number of ryegrass tillers/plant was 12.4 for SD2, but decreased to 5.0 for SD4 (*P*<0.01). The average number of ryegrass leaves/plant decreased from 16.9 to 14.8 (*P*<0.01) and tillers/plant decreased from 10.2 to 8.6 (*P*<0.01) as ryegrass seeding rate increased from 3 to 12 kg/ha.

Table 6.3 Seedling characteristics of Caucasian (CC) and white (WC) clovers and perennial ryegrass at the first defoliation date[1] after sowing on four sowing dates with four perennial ryegrass seeding rates.

	Plant population (number/m^2)		Shoot dry weight (mg)		Root dry weight (mg)		Number of leaves/ plant		Number of branches/ plant	
	Clover	Ryegrass	Clover	Ryegrass	Clover	Ryegrass	Clover	Ryegrass	Clover	Ryegrass
Clover (C)										
CC	51_b	123	39	671	24_a	156	4.3_b	16.0	0_b	9.6
WC	60_a	129	40	693	14_b	148	7.4_a	15.6	0.9_a	9.3
s.e.m.	2.0	2.0	2.4	10.6	1.1	5.9	0.17	0.28	0.09	0.24
P_C	0.006	0.061	0.986	0.149	<0.001	0.331	<0.001	0.372	<0.001	0.521
Seeding rate (R)										
0 kg/ha	62_a	0_d	38	–	17_b	–	6.0	–	0.5	–
3	60_a	79_c	43	739_a	22_a	168_a	6.1	16.9_a	0.7	10.2_a
6	54_a	149_b	41	689_b	19_{ab}	151_{ab}	5.9	15.9_{ab}	0.5	9.5_{ab}
12	46_b	275_a	36	618_c	17_b	137_b	5.4	14.8_b	0.4	8.6_b
s.e.m.	2.8	2.9	3.4	13.0	1.5	7.3	0.69	0.35	0.12	0.29
P_R	0.001	<0.001	0.421	<0.001	0.041	0.022	0.146	0.002	0.374	0.005
Sowing date (D)										
24 Sep.	73_a	106_b	44_a	758_b	21_a	155_a	6.0_a	15.9_c	0.8	9.4_b
9 Nov.	34_b	139_a	52_a	950_a	24_a	214_a	7.1_a	20.4_a	0.5	12.4_a
4 Feb.	77_a	138_a	48_a	696_b	20_a	163_a	6.1_a	17.4_{bc}	0.5	11.0_{ab}
31 Mar.	39_b	120_{ab}	14_b	324_c	10_b	75_b	4.1_b	9.8_d	0.2	5.0_c
s.e.m.	3.2	4.4	3.7	13.7	1.2	14.8	0.36	0.14	0.16	0.40
P_D	0.005	0.034	0.015	<0.001	0.013	0.026	0.034	<0.001	0.259	0.003
Interactions (P<0.05)	none	D×R D×C×R	none	D×R	none	none	D×C	none	none	none

Note: [1] Details of first defoliation dates are given in Section 6.2.3.
Within columns and main effects, values with the same or no letter subscript are not significantly different (α=0.05).

Table 6.4 Number of leaves/plant of Caucasian and white clovers at the first defoliation date[1] after sowing on four dates.

	Caucasian clover	White clover
	leaves/plant	
24 September	4.3_{ab}	7.7_{b}
9 November	4.8_{a}	9.4_{a}
4 February	4.7_{a}	7.6_{b}
31 March	3.4_{b}	4.8_{c}

Note: [1] Details of first defoliation dates are given in Section 6.2.3. Within columns, values with the same or no letter subscript are not significantly different ($\alpha = 0.05$).

6.3.5 Spring regrowth

Data for the botanical composition and total DM yield of each treatment at each harvest are reported in Appendices 15–18. This section compares the DM production rates of Caucasian and white clovers from the 3 kg/ha ryegrass seeding rate which produced the highest clover content to examine the effect of sowing date.

For SD1, clover growth rate at first defoliation was <2 kg/ha/d for both species (Figure 6.4). White clover growth then increased to 23 kg/ha/d in February and then decreased to 3 kg/ha/d in June before increasing again to 20 kg/ha/d on 6 November 2000. Caucasian clover growth was about 7–12 kg/ha/d lower between January and May, and about 16 kg/ha/d lower on 6 November 2000. For SD2, both species produced <5 kg DM/ha/d from first defoliation in January to August 2000, but white clover growth rate then increased to 15 kg/ha/d compared with 4 kg/ha/d for Caucasian clover on 6 November 2000. Similarly, for SD3 both species produced <4 kg/ha/d from first defoliation on 2 April to August 2000, but white clover growth rate then increased to 20 kg/ha/d compared with only 1 kg/ha/d for Caucasian clover on 6 November 2000. For SD4, clover growth rate was <5 kg DM/ha/d for both species.

6.3.6 Nitrogen % in ryegrass herbage

Mean N concentration in ryegrass herbage averaged 2.3% from the 24 September sowing compared with 3.3% for the 31 March sowing. N concentration in ryegrass herbage averaged 2.6% with both 3 and 12 kg/ha of ryegrass regardless of clover species.

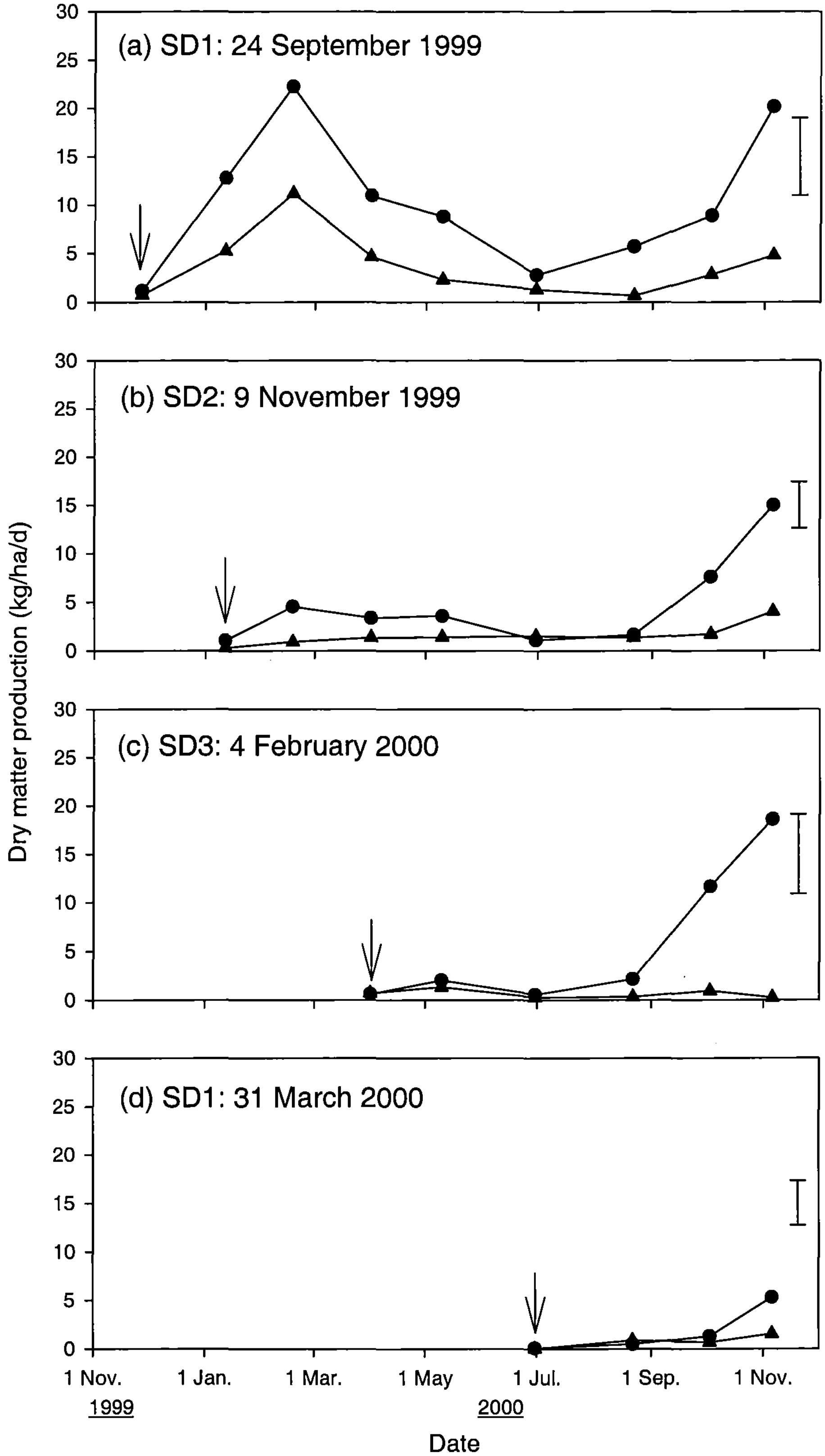

Figure 6.4 Dry matter production rate of Caucasian (▲) and white (•) clovers sown on 24 September 1999 (a), 9 November 1999 (b) and 4 February 2000 (c) with 3 kg/ha of perennial ryegrass. Bars represent maximum standard errors for final harvest. Arrows indicate first defoliation.

6.4 Discussion

6.4.1 White clover establishment

6.4.1.1 Spring sowing dates

Botanical composition (Figure 6.2) and total DM production (Table 6.1) measured on 6 November 2000 show that white clover establishment in spring was successful from the 24 September and 9 November sowing dates with 3 to 12 kg/ha of perennial ryegrass. Specifically, acceptable white clover content (>15%) and weed suppression (≤2%) was achieved from both spring sowing dates. Heavy weed ingress in the September sown pastures was controlled by the use of herbicides (Section 6.2.3), but weed suppression by perennial ryegrass without herbicides was effective in the November sown pastures.

Acceptable white clover and weed contents were achieved with 3–12 kg/ha of perennial ryegrass with minimum impact on total yield. All ryegrass combinations produced about 15–16 t DM/ha from the 24 September sowing date and about 13–15 t DM/ha from the 9 November sowing date when pastures were harvested in the following spring (Table 6.1). The greatest yields were obtained from treatments sown with 6 and 12 kg/ha of ryegrass, but the small (1–2 t DM/ha) difference between these treatments and the 3 kg/ha ryegrass treatment meant that the yields from all ryegrass treatments were acceptable for successful establishment of white clover. This result is consistent with Dumbleton (1997) who reported only small differences in total yield with 4–16 kg/ha of perennial ryegrass.

6.4.1.2 Autumn sowing dates

The impact of perennial ryegrass competition on the establishment of white clover was highlighted by the two autumn sowing dates. Only white clover sown on 4 February achieved adequate levels (≥13%) when mixed with perennial ryegrass, and all combinations suppressed weeds (0%) with minimum impact on total yields (8–9 t DM/ha) by the following spring (Figure 6.3; Table 6.1). The dominance of perennial ryegrass was accentuated in the late autumn (31 March) sowing, with no ryegrass treatment producing more than 8% white clover in the subsequent spring composition, even when the ryegrass seeding rate was only 3 kg/ha. This result is consistent with Moot *et al.* (2000) who recommended white clover based pastures are sown when autumn soil temperatures at 20 mm soil depth are at least 14 °C. The suppression of white clover by winter annual weeds in the clover monoculture sown on 31 March, when soil and air temperatures were declining rapidly below 10 °C (Figure 6.1), supports this conclusion. Thus, the establishment of white clover in autumn with perennial ryegrass was only successful from the 4 February sowing date with the inclusion of 3 to 12 kg/ha of perennial ryegrass.

6.4.2 Caucasian clover establishment

6.4.2.1 Spring sowing dates

The sowing strategies that resulted in successful establishment of white clover with perennial ryegrass did not achieve the same results for Caucasian clover. The inclusion of perennial ryegrass suppressed weeds (Figure 6.2) and maximised total yields (Table 6.1) effectively, but this was at the expense of Caucasian clover establishment. Indeed, even when sown on 24 September with the very low ryegrass seeding rate of 3 kg/ha, only 9% of the botanical composition was Caucasian clover in the following spring. In comparison, the same agronomic treatment achieved more than double the amount of white clover (28%). The warmer soil temperatures for the 9 November sowing date (Figure 6.1) should have benefited Caucasian clover establishment (Moot *et al.*, 2000). However, insect damage in the first month after sowing reduced plant populations (Table 6.3) which would have compromised these results. Only the clover monocultures achieved acceptable levels of Caucasian clover (47–61%) from both spring sowing dates. However, the establishment of the clover monoculture was unacceptable overall, with more weeds and less total yield (Table 6.1) than the ryegrass mixtures. Thus, spring sowing aided Caucasian clover establishment, but it was sensitive to even low (3–12 kg/ha) ryegrass seeding rates.

6.4.2.2 Autumn sowing dates

In contrast to white clover establishment, none of the treatments sown in autumn resulted in acceptable establishment of Caucasian clover. Indeed, even when sown on 4 February, when air and soil temperatures were above 16 °C (Figure 6.1), and with no ryegrass only 36% of the botanical composition of the monoculture in the following spring was Caucasian clover. The remainder was summer and winter annual weeds (Figure 6.3). The lack of competitive ability of Caucasian clover was apparent with the inclusion of ryegrass reducing the clover content to less than 3% of the total yield. Thus, the sowing strategies that achieved successful establishment of white clover did not assist Caucasian clover establishment.

6.4.3 Seedling growth and development

The seedling growth and development characteristics of white and Caucasian clovers offer some insight into the difference in establishment success between the two species.

6.4.3.1 Emergence

The thermal time to emergence was similar for white and Caucasian clovers, indicating that emergence was not the reason for poor establishment of Caucasian clover. Widdup *et al.* (1998) also reported similar emergence for white and Caucasian clovers. However, emergence is only one aspect of seedling competitiveness. After emergence, the ability of a species to compete for limited resources, particularly light, is dependent on its seedling growth and development characteristics.

6.4.3.2 Seedling growth

Caucasian clover seedlings showed active growth in the early stages, producing similar shoot dry weights than white clover at first defoliation (Table 6.3). However, the DM production after first defoliation was lower for Caucasian clover than white clover (Figure 6.4). This was despite adequate populations at first defoliation, which suggests slower seedling regrowth than white clover.

The root dry weights of white and Caucasian clovers were different. Specifically, Caucasian clover allocated 0.60:1 proportion of carbon to root and shoot growth compared with 0.37:1 for white clover. Thus, two species-specific patterns of carbon allocation were operating as early as first defoliation. This result is consistent with other Caucasian clover establishment studies. Specifically, researchers have attributed low production of Caucasian clover in the establishment year to its priority toward root and rhizome production. Hill and Mulcahy (1995) reported that the root/shoot ratio of Caucasian clover averaged around 3.0 at the end of their 1 year glasshouse experiment, and has been shown to be a characteristic of old stands (Daly and Mason, 1987; Stewart and Daly, 1980). In the present study, root and rhizome production beyond the first defoliation was not measured, but it is likely that a greater priority toward root than shoot production contributed to the slower DM production rate (Table 6.4) of Caucasian clover compared with white clover in the establishment year. However, root/shoot ratio may not have been the only factor contributing to the slow establishment of Caucasian clover.

6.4.3.3 Seedling development

There were also differences in seedling development between the two clover species. By first defoliation, white clover plants averaged 7.4 leaves compared with about 4 leaves for Caucasian clover (Table 6.3). This difference in leaf development was attributed to white clover plants that had initiated axillary buds that were beginning to grow out into stolons. In contrast, Caucasian clover showed no sign of crown shoot development and therefore produced fewer leaves than white clover. Thus, Caucasian clover appeared to have a slower rate of leaf and shoot development than white clover. This feature would place Caucasian clover at a disadvantage against perennial ryegrass after defoliation, due to fewer growing points from which leaves could be produced for light interception. White clover demonstrated early shoot development and therefore more growing points for leaf development, giving greater shoot regrowth after defoliation.

6.4.4 Spring regrowth

The consequence of the early differences in shoot development between Caucasian and white clovers was highlighted during the first spring after sowing. By November 2000, white clover averaged ~20 kg DM/ha/d when sown on the first three sowing dates with 3 kg/ha of perennial ryegrass, compared with 5 kg DM/ha/d or less for Caucasian clover. This result was probably due to stolon proliferation in white clover once the seedlings had established (Toddhunter, 1997). In contrast, it is likely that Caucasian clover had a slower rate of growing point development than white clover during the first spring which resulted in lower clover production rates. Previous research has reported a slower rate of growing point development and production than white clover during the establishment year (Watson *et al.*, 1996), but greater production from Caucasian clover during the second year in perennial ryegrass-based pastures (Watson *et al.*, 1997).

6.4.5 Implications for white clover establishment

The inference from the present study is that white clover with perennial ryegrass should be sown in spring, when soil temperatures are above 12 °C, or in early autumn, when soil temperatures are above 16 °C with low to medium (3–12 kg/ha) ryegrass seeding rates. This is achievable with irrigation and adequate rainfall, but may be difficult to achieve in dryland situations on the east coast of New Zealand. For example, in Canterbury a successful late summer sowing of white clover may only be achievable in only 1 out 4 years which is the general ratio of moist:dryland growing seasons in this environment.

This study has shown that as soil temperatures declined in the autumn (Table 6.1) the rapid seedling growth and development of perennial ryegrass favoured its establishment at the expense of white clover. Therefore, if sowing is delayed in the autumn by lack of soil moisture then white clover establishment will be less successful. In these conditions, early spring sowing should be the strategy used to enable seedlings to become established before moisture becomes limiting. The seeds will be sown into a moist but cool (>12 °C) seedbed but when soil temperatures are increasing.

The 12 kg/ha ryegrass seeding rate used in this study gives an indication of how clover content would decline even more if commercially recommended ryegrass sowing rates of 20–25 kg/ha are used. Cullen (1958) demonstrated emphatically the effects of high ryegrass sowing rates on slow establishing pasture species and concluded that low (4–10 kg/ha) rates of ryegrass were needed if seedlings of slower-establishing, small-seeded species such as cocksfoot, timothy and white clover were to be encouraged.

6.4.6 Implications for Caucasian clover establishment

These agronomic strategies which resulted in successful establishment of white clover did not result in successful establishment of Caucasian clover. The low Caucasian clover production from all ryegrass sowing rate combinations adds to its reputation as a difficult species to establish in pastures (Section 2.5).

The results from this study led to the conclusion that for successful establishment Caucasian clover needs to be sown alone in spring. This is an unusual requirement for permanent pasture establishment so a strategy acceptable to farmers could be to sow a cover crop such as rape with Caucasian clover. Indeed, successful rapid Caucasian clover establishment in perennial ryegrass pastures has usually been achieved from spring sowing with temporal separation of the two species. Other strategies required for rapid establishment of Caucasian clover will be discussed in more detail in Chapter 8.

6.5 Conclusions

This experiment has evaluated agronomic sowing strategies for the establishment of white and Caucasian clovers in permanent pastures. Specific conclusions were:

1. White clover establishment in spring was successful from the 24 September and 9 November sowing dates, when soil temperature was above 12 °C, and when ryegrass seeding rate was 3 to 12 kg/ha.
2. White clover establishment in autumn was only successful from the 4 February sowing date, when soil temperature was above 16 °C, and when ryegrass seeding rate was 3 to 12 kg/ha.
3. Effective weed suppression and similar total DM yields were achieved with 3 to 12 kg/ha of perennial ryegrass from all four sowing dates.
4. Caucasian clover establishment in spring was unsuccessful, from both the 24 September and 9 November sowing dates, when soil temperature was above 12 °C, and even when ryegrass seeding rate was restricted to 3 kg/ha. Only Caucasian clover sown alone in spring produced high clover contents (>60%).
5. None of the sowing strategies used in autumn resulted in successful establishment of Caucasian clover.
6. Seedling emergence was similar for white and Caucasian clovers, but post-emergence seedling growth and development differed. These results indicated a need for further detailed studies to help understand the physiological basis of slow establishment in Caucasian clover.

Chapter 7

Seedling development and growth of Caucasian clover, white clover and perennial ryegrass

7.1 Introduction

In Chapter 6, results showed that the successful establishment of white clover was achieved from spring and late summer sowing dates with low (3–12 kg/ha) seeding rates of perennial ryegrass. However, none of the sowing strategies tested resulted in rapid establishment of Caucasian clover with perennial ryegrass. Only Caucasian clover sown in spring without perennial ryegrass had achieved adequate clover contents in the following spring.

The identification of characteristics responsible for the slow establishment of Caucasian clover may provide a basis for understanding seedling competitiveness, and could assist the development of alternative sowing strategies. Important seedling characteristics include rapid germination, emergence and canopy expansion to compete for limited resources, particularly light (Brougham, 1953). The major components of seedling canopy expansion include the rate of leaf appearance (phyllochron) on the primary stem and timing of axillary shoot development (branch or tiller structures). Assuming development rate is linearly related to temperature up to an optimum (Angus *et al.*, 1981) then each development stage can be quantified using thermal time (Arnold and Monteith, 1974). Moot *et al.* (2000) used thermal time to quantify differences in germination and emergence of temperate pasture species including white clover and perennial ryegrass, but not Caucasian clover. Hill and Luck (1991) reported the effect of temperature on germination and seedling growth and development of Caucasian and white clovers, but Tt requirements were not reported.

Thus, the initial objective of the experiments described in this chapter was to quantify the Tt requirements for key seedling development stages (germination, emergence, first leaf appearance, phyllochron and axillary shoot development) of Caucasian clover, white clover and perennial ryegrass. The second objective aimed to relate differences in field establishment success to the development stages and additional growth characteristics (leaf area, seedling dry weight, shoot relative growth rate) of each species. This was done through a series of controlled environment experiments.

7.2 Materials and methods

7.2.1 Experiment 1: Germination

For Experiment 1, three replicates of 50 seeds per species ('Endura' Caucasian clover, 'Grasslands Demand' white clover and 'Nui' perennial ryegrass) were placed on wet blotting paper in Petri dishes and germinated in unlit incubators at 15 constant temperatures, ranging from 5.0 to 40.0 °C (±1°C) at 2.5 °C intervals. Clover seeds were scarified but no other pre-conditioning treatments were used. Distilled water was added as required to ensure moisture was non-limiting for germination.

Normal seedlings (International Seed Testing Association, 1985) were counted and removed daily at temperatures below 15 °C and twice daily at 15 °C or above, until germination ceased. Gompertz functions were fitted to cumulative percentage germination against days:

Equation 7.1 $$p = a*\exp(-\exp[-b*(t - m)])$$

where p is the cumulative percentage of seeds germinated at time t (d), a is the final germination percentage, and b and m are constants. The number of days to 75% germination (t_{75}) was calculated using a formula derived from the Gompertz function:

Equation 7.2 $$t_{75} = m - \ln[-\ln(75)]/b$$

7.2.2 Experiments 2–6: Seedling development and growth

7.2.2.1 Experimental design

Experiments 2–6 used a completely randomised design consisting of three species with three replicates, but each experiment was conducted in growth cabinets at five different nominal temperature settings (9/4, 15/5, 20/10, 25/15, or 30/20 °C).

7.2.2.2 Husbandry

Plastic 4 litre pots were filled with bark and pumice (4:1 by volume) below a 20-mm layer of loam and peat (1:1 by volume). The growing medium was amended with 1 g/l Osmocote Plus (15% N, 5% P, 11% K), trace elements and 1 g/l of dolomite lime (11% Mg, 24% Ca). In each pot, 50 seeds of the same lines as in Experiment 1 were placed on a 10 mm layer of loam and peat and then covered with another layer to achieve a sowing depth of 10 mm.

Immediately after sowing, the pots were watered and placed in a plant growth chamber (Conviron PGV36, Winnipeg, Canada) at the designated nominal temperature. An 8 h/8 h temperature and photoperiod regime was used, with 4 h transitions between day and night. Each chamber was lit with a combination of 45 incandescent (Sylvania, 40 W) and 30 fluorescent (Sylvania, 6 x 115 W and 24 x 215 W) lamps. Light reaching the plant canopies had a photosynthetic photon flux density (PPFD) of 350 ± 50 μmol/m^2/s. Relative humidity ranged from 50 to 70%. Pots were watered daily and re-randomised weekly to minimise possible effects of uneven air or light distribution within the chambers.

Temperatures were measured using four sensors (Thermistors KTY-110). Directly after sowing, two soil temperature sensors were placed in two pots, at a depth of 10 mm. Another two sensors (covered with aluminium foil) were placed in the same pots, located 10 mm above the soil surface, to be in close proximity to the seedling apical growing points, the major site of temperature perception by the plant (Peacock, 1975). Such placement has been recommended for accurate prediction of leaf appearance rates in crops (Jamieson *et al.*, 1995). Temperatures were recorded every 5 minutes and integrated and logged every hour with a HOBO data logger to determine daily mean temperatures. Because of small variations between nominal chamber temperature settings and the actual temperatures the plant experienced, the latter were used for all analyses. Specifically, 10 mm soil temperatures were used for emergence, but further seedling development was related to the 10 mm above-soil surface temperatures.

Plants were repeatedly thinned to avoid competition. Final populations were 10 plants/pot (390 plants/m^2). These plants were marked with coloured wire for detailed monitoring.

7.2.2.3 *Measurements*

7.2.2.3.1 Emergence

Emerged seedlings were recorded daily until seedlings ceased to emerge. Emergence was considered to have occurred when both cotyledons of the clover species had expanded and when the coleoptile of ryegrass was visible (Moot *et al.*, 2000). Gompertz functions (Equation 7.1) were fitted to cumulative percentage emergence against days after sowing (DAS). The number of days to 50% emergence (t_{50}) was calculated using a formula derived from the Gompertz function:

Equation 7.3 $$t_{50} = m - \ln[-\ln(50)]/b$$

7.2.2.3.2 Leaf appearance

The number of emerged leaves (unifoliate and trifoliate leaves in clovers) on the marked plants was recorded daily until individual Caucasian clover plants had produced five leaves on the primary stem (up to 774 °Cd). Leaves were considered to have emerged as soon as the petiole of clovers was visible and when ryegrass leaves had formed a collar (Hsu and Nelson, 1986b). The leaf appearance interval (days/leaf) for those that appeared on the primary stem was determined by least squares regression analysis of cumulative leaf number against DAS.

7.2.2.3.3 Axillary shoot development

The initiation of axillary leaves and branch (stolons or crown shoots) or tiller structures, which emerged in the axils of primary leaves, was also recorded. For white and Caucasian clovers, a stolon or crown shoot was considered to have formed when two leaves had emerged on that axillary bud. At this point, the axillary bud was beginning to grow out into a stolon or crown shoot. For ryegrass, a tiller was considered to have formed when one leaf on that axillary bud had formed a collar.

7.2.2.3.4 Seedling growth

When individual Caucasian clover plants had produced one leaf (the unifoliate leaf), the shoots of three unmarked plants in each pot were removed at soil level and their dry weight (DW) determined.

For most treatments, white clover and perennial ryegrass plants were large (beyond the seedling stage), and beginning to compete within pots approaching 774 °Cd. Thus, all 10 marked plants in each pot were destructively harvested at this time. The plants were washed and separated into shoots and roots. The number of leaves and branch or tiller structures was recorded, and leaf area/plant was measured using a LI-COR model LI-3100 leaf area meter before the DW of shoots and roots was determined.

Shoot relative growth rate (RGR; mg/mg/d) was calculated from the logarithm of individual seed weight and mean seedling shoot DW for each species when Caucasian clover plants had produced one leaf:

Equation 7.4 $$RGR = (\ln W2 - \ln W1)/(t2 - t1)$$

where W1 is the seed fresh weight at 0 DAS ($t1$) and W2 is the shoot DW at the time of harvest ($t2$).

Individual seed weight (uncoated) was 0.63 mg for white clover, 2.20 mg for Caucasian clover and 2.00 mg for ryegrass.

Also, shoot RGR was calculated by Equation 7.4 with mean shoot DW at $t2$ and mean shoot DW at the time of final harvest ($t3$).

7.2.3 Experiment 7: Axillary shoot development

Experiment 7 was conducted at 25/15 °C and used a randomised complete block design with three replicates of each species. This experiment used the same seed lines and husbandry as Experiments 2–6, but white clover and perennial ryegrass plants were harvested 620 °Cd after sowing, and Caucasian clover plants were harvested 1400 °Cd after sowing. Thus, more rigorous thinning than that used in Experiments 2–6 was used to avoid inter-plant competition. Leaf appearance rate and the timing of stolon or crown shoot initiation in clovers were recorded using the methods described in Section 7.2.2.3. For perennial ryegrass, leaves were considered to have emerged as soon as the leaf tip was visible.

7.2.4 Data analysis

Data for each species were plotted as the reciprocal of the duration (in days) to each development stage (75% germination, 50% emergence, first leaf appearance, leaf appearance interval, initiation of axillary leaves and branch or tiller structures) against the mean temperature. The inverse of duration (1/d) represents the development rate. Least squares regression analysis was used for the positive linear portion of the response whereby:

Equation 7.5 $\quad$ Development rate = $a + bx$

The regression coefficients of the regression line were then related to T_b and Tt (Angus *et al.*, 1981; Moot *et al.*, 2000) as:

Equation 7.6 $\quad$ $T_b = -a/b$

and

Equation 7.7 $\quad$ $Tt = 1/b$

The optimum temperature (T_{opt}) for development was identified as the temperature above which development rate did not increase. For germination, T_{opt} was calculated as a point estimate from the interception of the regression line fitted to the increasing portion of the response, and a second regression line fitted to the decreasing portion (Roman *et al.*, 1999), using the coefficients of the two regression equations:

Equation 7.8 $$T_{opt} = (a_2 - a_1)/(b_1 - b_2)$$

where a_1 and a_2 are the intercepts of Regressions 1 and 2, respectively, and b_1 and b_2 are the slopes of Regressions 1 and 2, respectively.

Data for temperatures above T_{opt} were excluded from the analysis of thermal time (Angus *et al.*, 1981; Moot *et al.*, 2000). Rates of germination decreased rapidly to zero as temperature was increased above T_{opt} to the maximum temperature (T_{max}).

Maximum standard errors (s.e.) or standard errors of the mean (s.e.m.) are reported for each measured variable. Also, maximum 95% confidence intervals (c.i.) are reported for T_b, T_{opt} and T_{max} (Sokal and Rohlf, 1981) as used by Roman *et al.* (1999). Final emergence percentage, seedling development and growth characteristics at harvest were analysed using one-way ANOVA within each temperature treatment. Treatment means were compared using Fisher's protected l.s.d. test whenever the ANOVA indicated that differences among treatments presented $P<0.05$.

To enable direct comparison of the Tt requirements for each development stage between species, additional regression analyses were performed with T_b set at 0 °C (Moot *et al.*, 2000). This value of T_b was justified because the 95% confidence interval for the calculated T_b value encompassed 0 °C for all species and development stages, indicating that the two values were not significantly different.

7.3 Results

7.3.1 Experiment 1: Germination

7.3.1.1 *Final germination percentage*

Gompertz functions described the cumulative germination data ($R^2 \geq 0.97$) for each species up to 32.5 °C (Figure 7.1). However, an R^2 value of 0.92 for Caucasian and white clovers at 35 °C meant that the final germination % and the duration to 75% of final germination had to be estimated by interpolation. For each species, final germination was between 86 and 99% from 5 to 25 °C, but decreased to about 40% for Caucasian clover and perennial ryegrass, and only 6% for white clover at 35 °C. Germination was not observed for any of these species at 37.5 or 40 °C.

7.3.1.2 *Time to 75% germination and germination rate*

The number of days to 75% of the final germination was greatest at 5 °C for each species, but decreased exponentially ($R^2 \geq 0.99$) as temperature increased to 27.5 °C (Figure 7.2a). There was then an increase in duration until the maximum effective temperature of 37.5 °C was reached. White clover required 14 d to reach 75% germination at 5 °C compared with 18 d for Caucasian clover and 23 d for perennial ryegrass. At 27.5 °C, Caucasian and white clovers reached 75% germination in about 2 d compared with 3 d for perennial ryegrass.

Based on the fitted 'broken stick' model ($R^2 \geq 0.92$; s.e. ≤ 0.07) the estimated value of T_b for germination was ~3.3 °C for each species (Figure 7.2b). The minimum germination rate for each species was 0.06 seeds/d at the lowest temperature of 5 °C. This rate then increased to a maximum of ~0.69 seeds/d for both clovers compared with 0.38 seeds/d for ryegrass at the T_{opt} of ~27.1 °C, before it decreased until no germination occurred at the estimated T_{max} of 38.2 °C for all species.

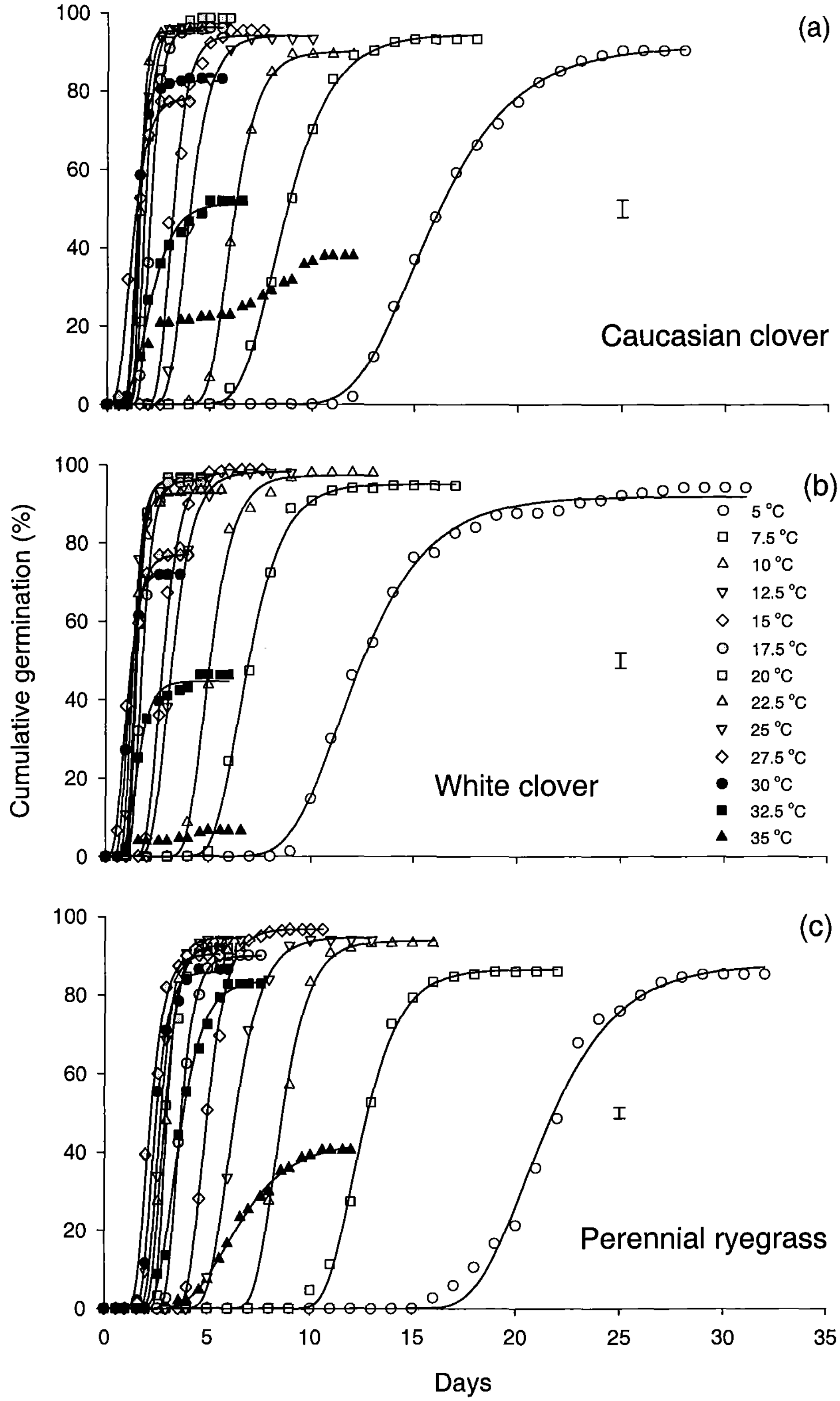

Figure 7.1 Cumulative germination of (a) Caucasian clover, (b) white clover and (c) perennial ryegrass at different constant temperatures. Error bars represent the maximum standard error for the final germination percentage.

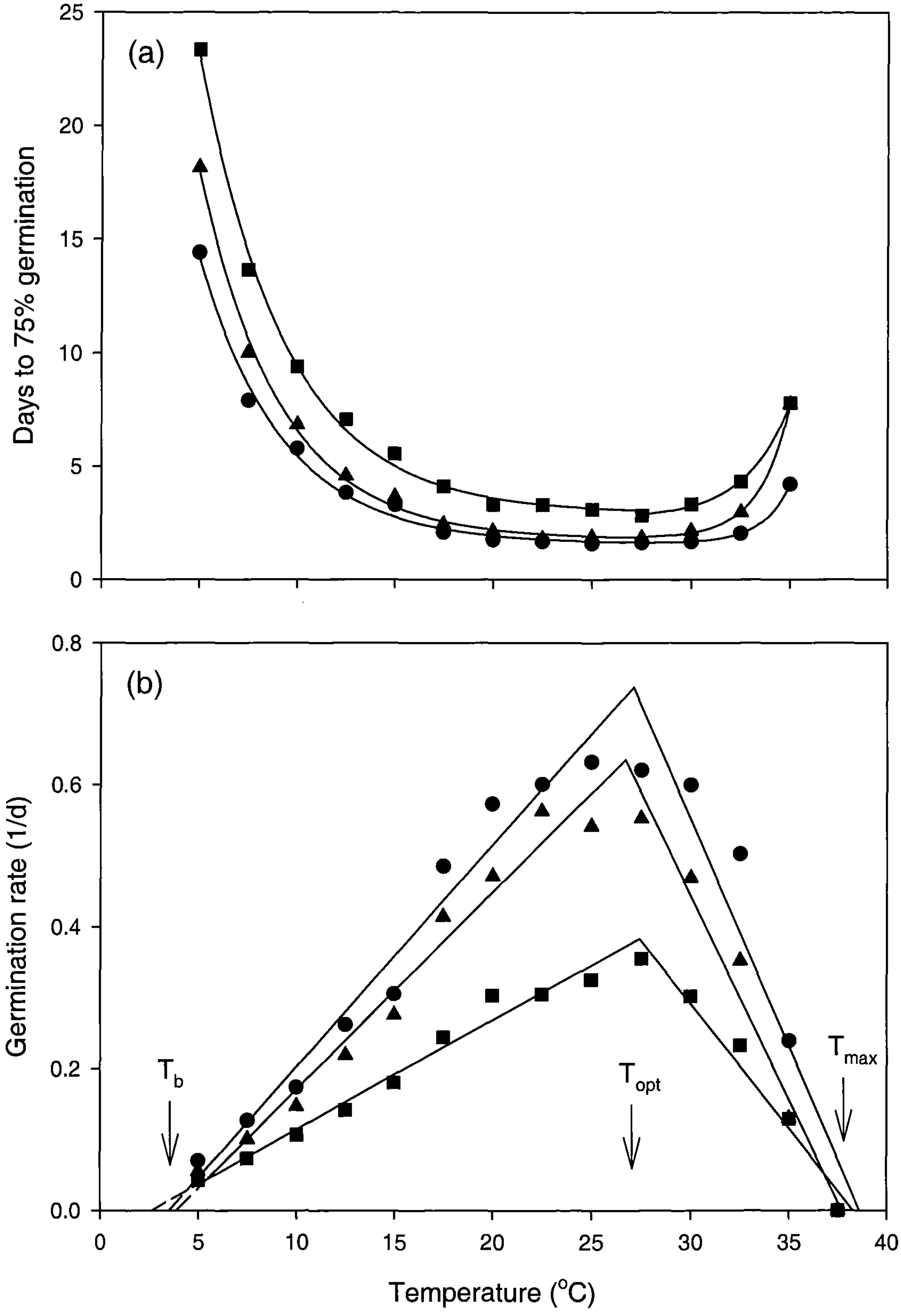

Figure 7.2 Number of days to 75% of final germination (a) and germination rate (b) of Caucasian clover (▲), white clover (●) and perennial ryegrass (■) at different temperatures. Arrows indicate base (T_b), optimum (T_{opt}) and maximum (T_{max}) cardinal temperatures for all three species.

The number of days to 75% germination differed as temperature increased (Figure 7.2a), but was constant in Tt when temperature was less than T_{opt} (Table 7.1). The Tt requirement for 75% germination was lowest for white clover at 32 °Cd compared with 36 °Cd for Caucasian clover and 65 °Cd for perennial ryegrass. When T_b was set at 0 °C, the two clovers still required about half the Tt of perennial ryegrass to reach 75% germination.

Table 7.1 Base (T_b), optimum (T_{opt}) and maximum (T_{max}) temperatures and thermal time (Tt) requirements for 75% germination of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR).

	T_b (°C)	T_{opt} (°C)	T_{max} (°C)	Tt (°Cd)	R^2 (%)	[1]Tt (T_b=0 °C) (°Cd)
CC	3.9	26.7	37.7	36	95	46
WC	3.5	27.1	38.6	32	92	40
PR	2.6	27.4	38.3	65	96	76
Max. s.e.	0.25	0.36	0.27	2.6		1.6
95% c.i.	-2.0, 6.8	24.8, 31.3	35.0, 50.3			

[1]Analysis assumes a base temperature of 0 °C.

R^2 = coefficients of determination.

7.3.2 Experiments 2–6: Seedling development and growth

7.3.2.1 Emergence

Gompertz functions described the cumulative emergence data ($R^2 \geq 0.99$) for each species and temperature treatment (Figure 7.3). For Caucasian clover, final emergence was between 71 and 78% from 10 to 25 °C, but decreased to 62% at 6.5 °C. For ryegrass, final emergence was between 79 and 87% from 6.5 to 25 °C, but for white clover emergence ranged between 65 and 77%.

The number of days to 50% of the final emergence was at least 24 d at 6.5 °C for each species, but decreased exponentially ($R^2 \geq 0.99$) to be about 4 d as mean 10-mm soil temperature increased to 25 °C (Figure 7.4a). This led to a linear ($R^2 \geq 0.97$) increase in the rate of emergence against mean soil temperature (Figure 7.4b) that enabled T_b and Tt to be estimated at about 3.4 °C and 92 °Cd respectively for all three species (Table 7.2). The calculated T_b was not significantly different to 0 °C, but the Tt requirement for emergence remained similar for all species when T_b was set at 0 °C.

Table 7.2 Base temperature (T_b) and thermal time (Tt) requirements for 50% emergence of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) grown in controlled environments.

	T_b (°C)	Tt (°Cd)	R^2 (%)	[1]Tt (T_b = 0 °C) (°Cd)
CC	3.6	92	99	114
WC	3.6	87	99	109
PR	3.0	96	98	115
Maximum s.e.	0.56	9.1		7.6
95% c.i.	-1.93, 8.79			

[1]Analysis assumes a base temperature of 0 °C.

R^2 = coefficients of determination.

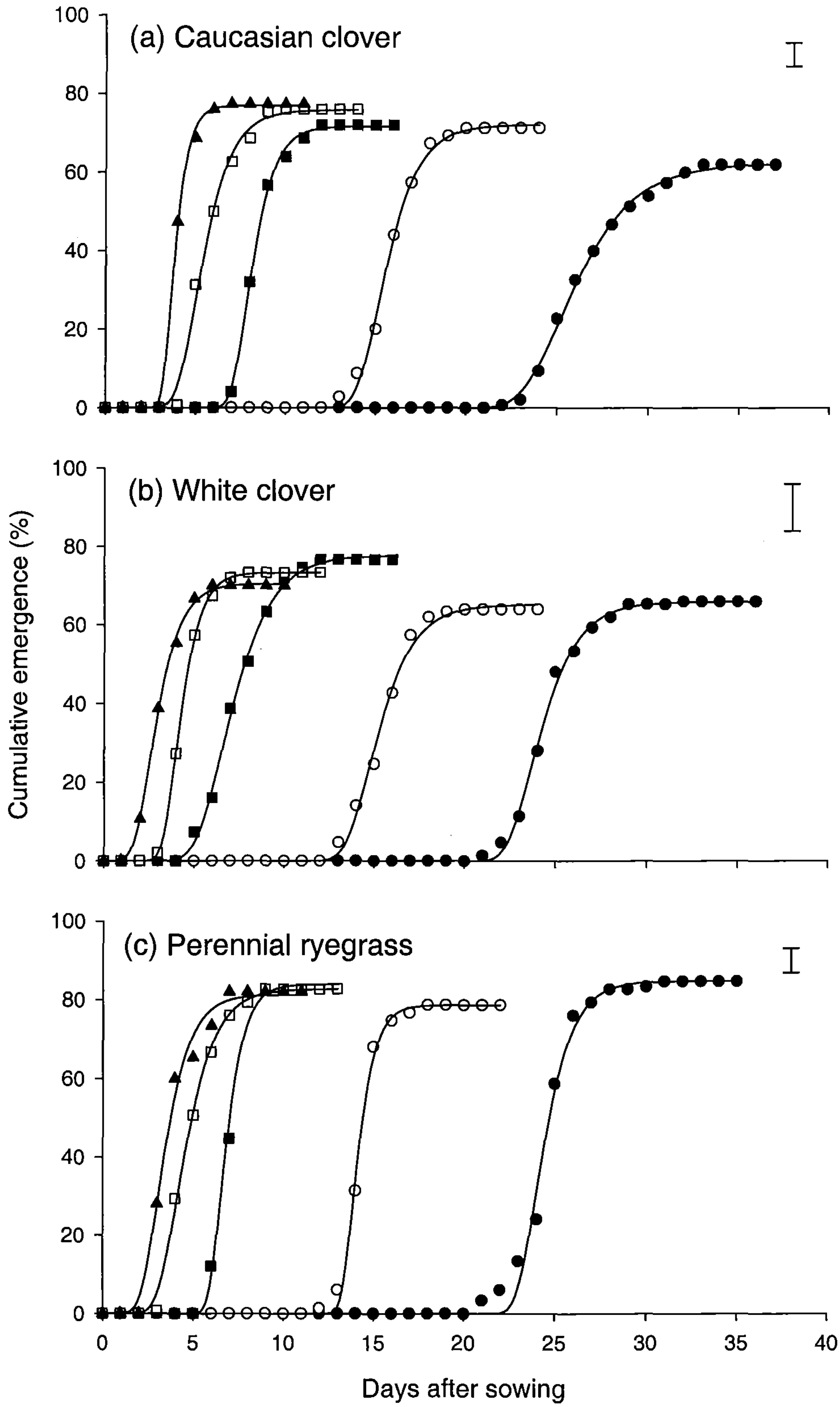

Figure 7.3 Cumulative emergence (%) of (a) Caucasian clover, (b) white clover and (c) perennial ryegrass at mean 10 mm soil temperatures of 6.5 (●), 10 (○), 15 (■), 20 (□) or 25 (▲) °C. Error bars represent the maximum standard error for the final emergence percentage.

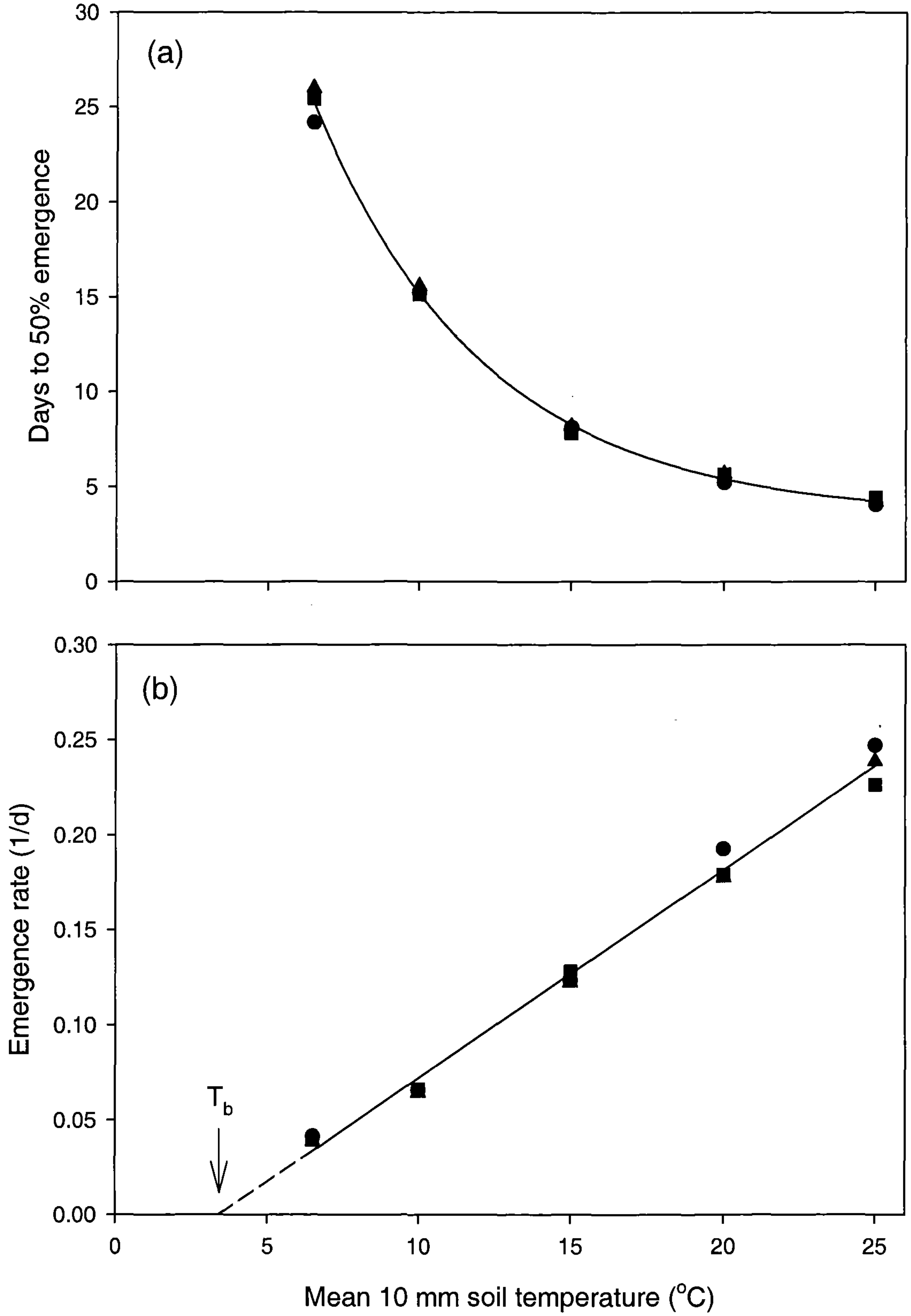

Figure 7.4 Number of days to 50% of final emergence (a) and emergence rate (b) of Caucasian clover (▲), white clover (●) and perennial ryegrass (■) at different temperatures.

7.3.2.2 First leaf appearance

The number of days to first leaf appearance (unifoliate leaf in clovers) was ~55 d for all species at 5.5 °C, but decreased exponentially ($R^2 \geq 0.99$) to be about 10 d as air temperature increased to a mean of 24 °C (Appendix 19a). Linear relationships ($R^2 \geq 0.93$) between first leaf appearance rate and mean air temperature from 5.5 to 19 °C (Appendix 19b) enabled T_b to be estimated at 3.4 °C for all species (Table 7.3). The Tt for first leaf appearance averaged 153 °Cd for Caucasian and white clovers compared with 180 °Cd for perennial ryegrass, and species differences did not change when $T_b = 0$ °C.

Table 7.3 Base temperature (T_b) and thermal time (Tt) requirements for first (unifoliate) leaf appearance of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) grown in controlled environments.

	T_b (°C)	Tt (°Cd)	R^2 (%)	[1]Tt (T_b = 0 °C) (°Cd)
CC	3.7	151	95	205
WC	3.6	155	93	208
PR	3.0	180	98	228
Maximum s.e.	0.51	6.2		8.4
95% c.i.	-6.91, 4.29			

Note: Data from 30/20 °C were excluded from the analysis of thermal time on the basis that these were above the species optimum thermal range (Section 7.2.4). [1]Analysis assumes a base temperature of 0 °C. R^2 = coefficients of determination.

7.3.2.3 Phyllochron

The number of leaves on the primary stem increased linearly with DAS for each species and temperature regime (Figure 7.5). The primary leaf appearance interval (days/leaf), or phyllochron, was greater than 18 d at 5.5 °C for each species, but decreased exponentially ($R^2 \geq 0.99$) to be ~5 d as air temperature increased to a mean of 19 °C (Appendix 20a). Leaf appearance rate (leaves/d) was a linear function ($R^2 \geq 0.99$) of mean air temperature from 5.5 to 19 °C (Appendix 20b) that enabled T_b to be estimated at ~1 °C for all species (Table 7.4). Above the calculated T_b, the phyllochron was 88 °Cd for white clover compared with ~98 °Cd for Caucasian clover and perennial ryegrass, and differences were similar when T_b = 0°C.

Table 7.4 The phyllochron of successive primary stem leaves above a base temperature (T_b) for Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) grown in controlled environments.

	T_b (°C)	Phyllochron (°Cd/leaf)	R^2 (%)	[1]Tt (T_b = 0 °C) (°Cd)
CC	1.4	98	99	109
WC	0.8	88	99	94
PR	0.5	97	99	101
Maximum s.e.	0.45	4.6		1.7
95% c.i.	-7.19, 3.32			

Note: Data from 30/20 °C were excluded from the analysis of thermal time on the basis that these were above the species optimum thermal range (Section 7.2.4). [1]Analysis assumes a base temperature of 0 °C. [2]Phyllochron was calculated from the spade leaf (primary leaf one) to five primary leaves in each species. R^2 = coefficients of determination.

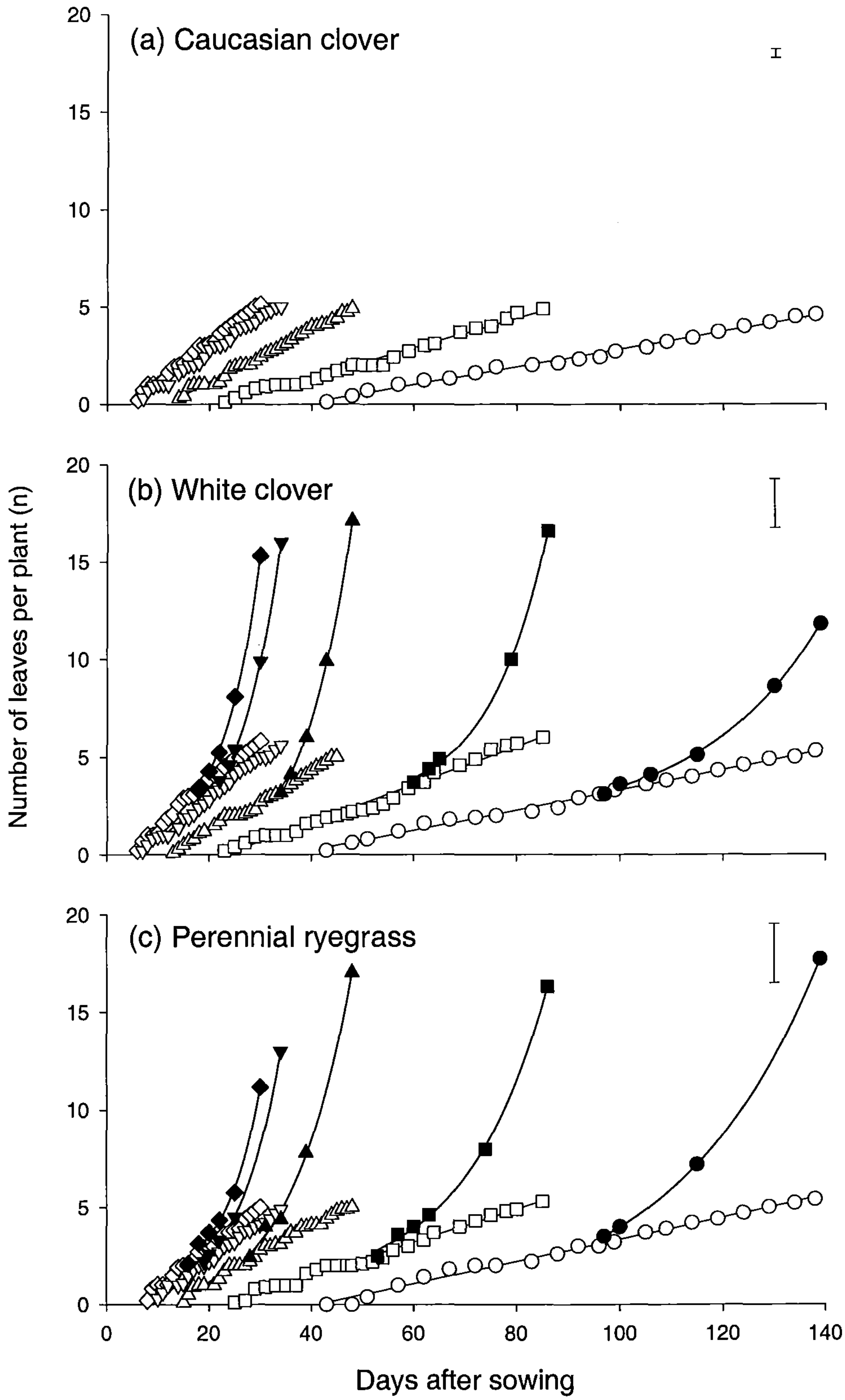

Figure 7.5 Number of total (closed symbols) and primary stem (open symbols) leaves of (a) Caucasian clover, (b) white clover and (c) perennial ryegrass plotted against days after sowing at a mean air temperature of 5.5 (●), 9 (■), 14 (▲), 19 (▼) or 24 (♦) °C. Error bars represent the maximum standard error for final total leaf number.

7.3.2.4 Initiation of axillary leaves and branch or tiller structures

Axillary leaves were initiated in perennial ryegrass and white clover when the third and fourth leaves appeared on the primary stem, respectively (Figure 7.5). The development of stolons in white clover and tillers in perennial ryegrass led to an exponential increase in the total number of leaves with DAS, whereas the number of leaves on the primary stem continued to increase linearly. Linear relationships ($R^2 \geq 0.95$) between axillary leaf (Appendix 21b), stolon or tiller (Appendix 22b) initiation rates and mean temperature from 5.5 to 19 °C were found in white clover and perennial ryegrass, and an average T_b of 2.2 °C was estimated (Table 7.5). With T_b set at 0 °C, ryegrass tillers initially appeared after 373 °Cd compared with 440 °Cd for stolons of white clover.

Table 7.5 Base temperature (T_b) and thermal time (Tt) requirements for the initiation of axillary leaves and stolons or tillers in white clover (WC) and perennial ryegrass (PR) grown in controlled environments.

	T_b (°C)	Tt (°Cd)	R^2 (%)	[1]Tt (T_b = 0 °C) (°Cd)
WC (axil. leaves)	1.5	362	97	440
WC (stolons)	2.5	477	99	532
PR*	2.7	301	99	373
Maximum s.e.	0.51	23.8		14.1
95% c.i.	-12.97, 2.44			

Note: Data from 30/20 °C were excluded from the analysis of thermal time on the basis that these were above the species optimum thermal range (Section 7.2.4). [1]Analysis assumes a base temperature of 0 °C. R^2 = coefficients of determination. *Axillary leaf and tiller initiation were deemed to be the same in perennial ryegrass.

In contrast, no axillary leaves, crown shoots or rhizomes were detected in Caucasian clover before five leaves had appeared on the primary stem (up to 774 °Cd). This necessitated Experiment 7 to accurately define the initiation of branch development in Caucasian clover (Section 7.3.3).

7.3.2.5 Seedling shoot dry weight at first leaf appearance, and shoot relative growth rate from seed to first leaf appearance

When Caucasian clover had produced the first primary leaf its shoot DW averaged 8.0 mg/plant compared with (P<0.05) 10.0 mg/plant for perennial ryegrass and 3.3 mg/plant for white clover (Table 7.6). However, shoot RGR from seed to first leaf appearance averaged 0.025 mg/mg/d for all species.

Table 7.6 Shoot dry weight and shoot relative growth rate of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) at different nominal temperatures at first leaf appearance in Caucasian clover.

	9/4 °C	15/5 °C	20/10 °C	25/15 °C	30/20 °C	Mean
Shoot dry weight (mg/plant)						
CC	11.8_b	11.0_a	7.6_a	5.7_a	4.0_a	8.0_b
WC	6.8_b	4.1_b	2.1_b	2.1_b	1.3_b	3.3_c
PR	18.9_a	14.0_a	9.2_a	4.9_a	3.1_a	10.0_a
s.e.m.	1.77	1.10	1.03	0.49	0.26	0.31
P	0.021	0.007	0.017	0.015	0.005	<0.001
Shoot relative growth rate (mg/mg/d)						
CC	0.011	0.023	0.024	0.031	0.025	0.023
WC	0.015	0.027	0.023	0.040	0.031	0.027
PR	0.015	0.028	0.030	0.030	0.018	0.024
s.e.m.	0.0011	0.0013	0.0024	0.0033	0.0036	0.0018
P	0.089	0.118	0.209	0.158	0.166	0.347

Note: Shoot relative growth rate was calculated from seed to first leaf appearance. Within columns and variables, values with the same or no letter subscript are not significantly different (α=0.05).

7.3.2.6 Number of leaves and branches, and leaf area/plant at final harvest

By 774 °Cd, the number of leaves/plant averaged 14 for white clover and perennial ryegrass compared with (P<0.001) only 5 for Caucasian clover at final harvest (Table 7.7). Perennial ryegrass averaged 7.3 tillers/plant compared with (P<0.001) 2.5 stolons/plant for white clover and no crown shoots for Caucasian clover. However, mean leaf area/plant was similar for Caucasian and white clovers at 24 cm^2 compared with (P<0.001) 82 cm^2 for perennial ryegrass.

Table 7.7 Number of leaves and branches/plant and leaf area/plant of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) at different nominal temperatures up to 774 °Cd after sowing.

	9/4 °C	15/5 °C	20/10 °C	25/15 °C	30/20 °C	Mean
Number of leaves/plant						
CC	4.7_c	5.1_b	5.4_b	5.0_b	5.1_c	5.1_b
WC	11.8_b	16.6_a	17.8_a	16.0_a	15.3_a	15.5_a
PR	17.7_a	16.3_a	17.0_a	13.0_a	11.2_b	15.0_a
s.e.m.	0.41	0.85	0.30	0.78	0.80	0.60
P	<0.001	0.001	<0.001	0.001	0.002	<0.001
Number of branches/plant						
CC	0_c	0_c	0_c	0_c	0_c	0_c
WC	2.1_b	2.7_b	2.9_b	2.5_b	2.4_b	2.5_b
PR	8.6_a	8.3_a	7.7_a	6.2_a	5.6_a	7.3_a
s.e.m.	0.24	0.41	0.13	0.29	0.16	0.19
P	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Leaf area/plant (cm^2)						
CC	17_b	25_b	29_b	25_b	21_b	24_b
WC	15_b	28_b	27_b	25_b	24_b	24_b
PR	84_a	107_a	82_a	72_a	64_a	82_a
s.e.m.	1.8	4.1	1.3	6.6	4.7	2.6
P	<0.001	<0.001	<0.001	0.011	0.005	<0.001

Note: Within columns and variables, values with the same letter subscript are not significantly different (α=0.05).

7.3.2.7 Seedling dry weight at final harvest

At 9/4 °C and 15/5 °C, perennial ryegrass had the greatest relative advantage (P<0.05) in shoot, root and total DW over Caucasian and white clovers (Figure 7.6). However, this advantage decreased with increasing temperature to be lowest at 30/20 °C. Shoot, root and total DW were similar for Caucasian and white clovers at all temperatures, but were lowest at 9/4 °C. Averaged across all temperatures, shoot DW was 172 mg/plant for both clovers compared with 551 mg/plant for perennial ryegrass, but root DW was 82 mg/plant for Caucasian clover compared with 62 mg/plant for white clover and 286 mg/plant for perennial ryegrass.

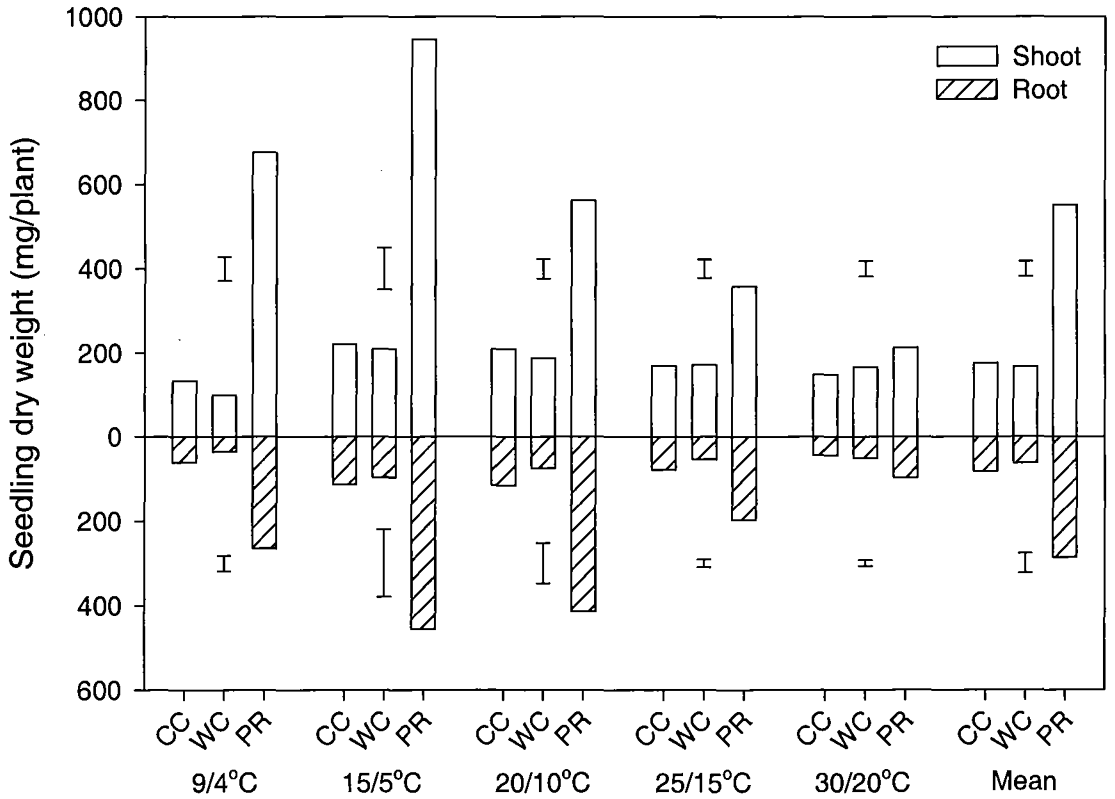

Figure 7.6 Individual shoot and root dry weights of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) at different nominal temperatures up to 774 °Cd after sowing. Error bars represent l.s.d. for means within each temperature regime.

7.3.2.8 Shoot relative growth rate from first leaf appearance to final harvest, and root/shoot ratio at final harvest

Average shoot RGR from first leaf appearance to final harvest for white clover and perennial ryegrass (~0.063 mg/mg/d) was higher (P<0.05) than Caucasian clover (0.049 mg/mg/d) at final harvest (Table 7.8). This result was consistent across all temperatures except at 9/4 °C where shoot RGR was similar for white and Caucasian clovers. Root/shoot ratios at final harvest were similar for all three species at 9/4 °C and 15/5 °C, but were highest for perennial ryegrass and lowest for white clover at the higher temperatures.

Table 7.8 Shoot relative growth rate from first leaf appearance and root/shoot ratio of Caucasian clover (CC), white clover (WC) and perennial ryegrass (PR) at different nominal temperatures up to 774 °Cd after sowing when all plants were harvested.

	9/4 °C	15/5 °C	20/10 °C	25/15 °C	30/20 °C	Mean
Shoot relative growth rate (mg/mg/d)[1]						
CC	0.015_b	0.023_b	0.056_b	0.070_b	0.079_c	0.049_b
WC	0.016_b	0.030_a	0.075_a	0.091_a	0.105_a	0.064_a
PR	0.021_a	0.033_a	0.069_a	0.089_a	0.092_b	0.061_a
s.e.m.	0.0006	0.0008	0.0021	0.0028	0.0021	0.0012
P	0.003	0.003	0.007	0.012	0.002	0.002
Root/shoot ratio						
CC	0.46	0.51	0.55_b	0.46_a	0.30_b	0.45_{ab}
WC	0.37	0.46	0.39_c	0.32_b	0.30_b	0.37_b
PR	0.39	0.49	0.73_a	0.56_a	0.46_a	0.53_a
s.e.m.	0.023	0.061	0.033	0.028	0.019	0.022
P	0.110	0.867	0.005	0.010	0.007	0.018

Note: Within columns and variables, values with the same or no letter subscript are not significantly different (α=0.05). [1] Shoot relative growth rate was calculated from when Caucasian clover had produced one primary leaf until final harvest for all three species.

7.3.3 Experiment 7: Axillary shoot development

Figure 7.7 shows the cumulative leaf appearance and initiation of axillary leaves and branch or tiller structures for each species grown at 25/15 °C accumulated Tt above a T_b of 0 °C. In this experiment, the accumulated Tt (± s.e.) to the initiation of tillers in perennial ryegrass was 360 ± 20 °Cd compared with 430 ± 31 °Cd for stolons in white clover. In contrast, axillary leaves were found in Caucasian clover only after 990 ± 28 °Cd and crown shoots after 1180 ± 42 °Cd. As a result, the total number of leaves produced per plant 620 °Cd after sowing was 18 for perennial ryegrass, 15 for white clover but only 4 for Caucasian clover. After 1400 °Cd, Caucasian clover had produced 16 leaves/plant compared with 76/plant for perennial ryegrass and 117/plant for white clover (s.e.m. = 5.1 leaves/plant). The phyllochron (± s.e.) for primary stem leaves (1–5) for white clover was 73 ± 2.3 °Cd compared with 91 ± 2.3 °Cd for ryegrass and 97 ± 6.1 °Cd for Caucasian clover.

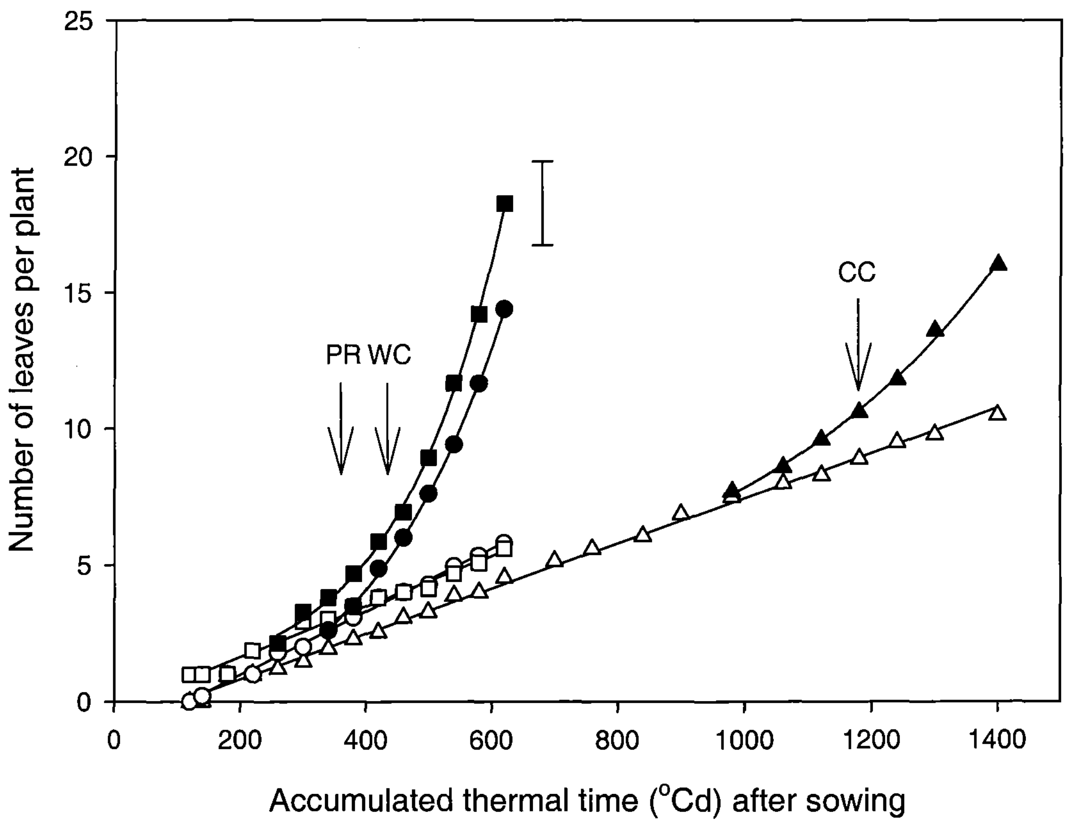

Figure 7.7 Number of total (closed symbols) and primary stem (open symbols) leaves of Caucasian clover (▲, CC), white clover (●, WC) and perennial ryegrass (■, PR) grown at 25/15 °C plotted against accumulated thermal time above a base temperature of 0 °C. Arrows indicate the initiation of tiller, stolon or crown shoot development. Error bar represents the standard error of the mean for total leaf number at 620 °Cd.

7.4 Discussion

7.4.1 Germination

The germination rates of all three species increased linearly with increasing temperature up to an optimum of about 27 °C (Figure 7.2b). The nature of the response of germination rate to sub-optimum temperatures was consistent with a proposed sigmoid or logistic response of development rate to temperature (Section 2.6.1.3). Moot *et al.* (2000) reported a similar linear decline from the optimum to a base temperature below which germination ceased. In the present experiment, a two-piece "broken stick" was appropriate for describing the sub- and supra-optimum responses of each species. The sub-optimum temperature range encompassed soil temperatures commonly experienced at spring and autumn sowing times in New Zealand (Charlton *et al.*, 1986). Thus, the discussion in this chapter focuses on utilising the positive linear portion of the response to determine Tt requirements for each species.

The final germination percentage of each species was above 80% for all temperatures in the sub-optimum range (Figure 7.1), indicating that no seed dormancy mechanisms were operating or additional pre-germination treatments were required. The duration to 75% germination was longer in days as temperatures decreased below the optimum (Figure 7.2a), but constant in thermal time. Hill and Luck (1991) reported similar responses to temperature for germination of Caucasian clover and 'Haifa' white clover, but their Tt requirements were not measured. In the present experiment, calculation of the Tt requirement for germination summarised the individual temperature responses of each species into repeatable coefficients that could be used for all temperatures in the sub-optimum temperature range. The similar Tt requirements for germination of Caucasian (36 °Cd) and white (32 °Cd) clovers confirm that the slow establishment of Caucasian clover observed in the field (Chapter 6) was not caused by slow germination.

The values of T_b determined for white clover and perennial ryegrass were within the range of values calculated for the same species in previous studies (Moot *et al.*, 2000; Trudgill *et al.*, 2000). However, the T_b values for Caucasian (3.9 °C) and white (3.4 °C) clovers differed from those reported (5.24 °C and 5.79 °C, respectively) by Hill and Luck (1991). This variation may have been caused by differences in the definition of "germination" or differences in cultivars and seed lines tested. Also, Hill and Luck (1991) used alternating temperatures whereas in the present study constant temperatures were used. This may be important if in the alternating temperatures the cooling down phase takes longer than the warming up phase, which will increase the mean temperature the seed actually experiences. In the present experiment, T_b was calculated from nine constant temperatures in the sub-optimum range, giving an accurate estimate of T_b for the prediction of Tt requirements for germination.

The thermal time requirements for germination were lower for Caucasian and white clovers than perennial ryegrass, which is consistent with Moot *et al.* (2000). The rapid germination of clovers may be attributed to a faster rate of inbibition (McWilliam *et al.*, 1970). Hampton *et al.* (1987) noted the faster germination of temperate herbage legumes than grasses and concluded that "...the time of sowing of seed mixtures will depend on the grass component". However, rapid germination does not guarantee successful establishment of a species. Rapid emergence, seedling development and growth are also important components of seedling competitiveness.

7.4.2 Emergence and first leaf appearance

The Tt requirements for emergence and first (unifoliate) leaf appearance were similar for all three species. This means that for soil temperatures commonly experienced in spring or autumn, Caucasian clover is expected to emerge and produce its first leaf at about the same time as white clover and perennial ryegrass.

Emergence was strongly correlated with first leaf appearance, but not with germination. Specifically, the two clover species, which germinated rapidly, did not emerge any faster than perennial ryegrass (Tables 7.1 and 7.2). This result may have been an artefact of differences in the definition of emergence between clovers (cotyledons expanded) and perennial ryegrass (coleoptile visible). The shoot relative growth rates from seed to first leaf appearance of the three species were similar (Table 7.6).

7.4.3 Phyllochron

The rate at which new leaves appeared on the primary stem differed slightly among species (Figures 7.5 and 7.7). This was quantified by the phyllochron, which was 88 °Cd for white clover compared with 98 °Cd for Caucasian clover and 97 °Cd for perennial ryegrass (Table 7.4). Five primary leaves appeared during the first month of seedling growth at a mean temperature of 19 °C in Caucasian clover and perennial ryegrass, and about six primary leaves appeared for white clover. The difference in phyllochron between the three species is therefore unlikely to be the main cause of slow establishment of Caucasian clover.

7.4.4 Axillary shoot development

The time to initiation of axillary shoot development was significantly different among the three species. At the time when the fifth leaf on the primary stem of all three species appeared there were also axillary leaves and buds beginning to grow into stolons in white clover and tillers in perennial ryegrass (Table 7.5). In contrast, Caucasian clover seedlings showed no sign of axillary leaf or crown shoot development in all treatments up to 774 °Cd after sowing. In Experiment 7, axillary leaf initiation on Caucasian clover occurred after about 990 °Cd, but Caucasian clover required 1180 °Cd for the initiation of crown shoot development (Figure 7.7). This compared with 430 °Cd for a stolon to appear in white clover and 360 °Cd for a tiller in perennial ryegrass. This meant that exponential leaf development began after 20 d of seedling growth at a mean temperature of 19 °C in perennial ryegrass compared with 23 d in white clover and 53 d in Caucasian clover. The consequence of these differences was shown by the number of leaves per plant which was three times more in ryegrass and white clover than in Caucasian clover up to 774 °Cd, and about five times more at 1400 °Cd after sowing. This lack of axillary leaf and crown shoot development indicates Caucasian clover would have poor competitive ability in mixed sowings.

Leaf appearance along the axis of the primary stem was driven by the accumulation of thermal time, enabling the phyllochron to be calculated. However, the differences in timing of axillary leaf and branch or tiller initiation may have been caused by differences in apical dominance between the three species. For example, in white clover axillary leaves were initiated after the appearance of the third primary leaf, suggesting that the first axillary bud on the primary stem was inactive until three primary leaves had appeared. In contrast, for Caucasian clover the first axillary bud was probably not released until seven leaves had appeared along the primary stem axis. In all three species, axillary leaf appearance was initiated only after a specific number of leaves had appeared on the primary stem, suggesting that apical dominance was strong in all species, and enabling this key development stage to be quantified using thermal time above a common T_b. Confirmation of the importance of apical dominance on the axillary bud development could be shown by removing the apex of the primary stem and quantifying when axillary leaf appearance starts.

7.4.5 Seedling growth after first leaf appearance

Despite the thermal time differences in axillary shoot development the shoot DW and leaf area of Caucasian and white clovers were not different up to 774 °Cd (Figure 7.6 and Figure 7.7). This result was probably an artefact of the timing of harvest in Experiments 2–6 at a maximum of 774 °Cd. During the additional 220 °Cd Caucasian clover would require to initiate axillary leaves, white clover would be producing leaves at an exponential rate. The slower rate of leaf development of Caucasian clover was in part compensated by its larger leaves (LAI values were equal) and higher leaf photosynthesis rate (Chapter 5).

The differences in development between the three species at 774 °Cd would have significant implications if the three species were defoliated or grazed at this time. For example, if all of the leaves were removed from ryegrass seedlings at 774 °Cd, the plants would have about eight growing points from which new leaves could be produced (Table 7.7). Thus, the total leaf appearance rate from ryegrass seedlings after grazing would be higher than that of white clover seedlings which had about three growing points per plant, and much higher than Caucasian clover seedlings which had no axillary branches and therefore only one growing point per plant. This suggests that early defoliation to reduce competition by ryegrass for light may not necessarily benefit the establishment of Caucasian clover seedlings, and adds greater reason for not sowing Caucasian clover in mixtures with ryegrass.

The root/shoot ratios gave some evidence of differences in carbon allocation above and below ground between white and Caucasian clovers up to 774 °Cd (Table 7.8). In Caucasian clover, carbon was allocated nearly equally to root growth and shoot production leading to an average root/shoot ratio of 0.45, whereas in white clover the balance was weighted toward shoot production, and the resultant root/shoot ratio averaged 0.37. Similar results have been frequently reported previously (e.g. Genrich *et al.*, 1998; Widdup *et al.*, 1998) and in the field experiment in Chapter 6 (Table 6.3). However, in the present study the differences were small, suggesting that the pattern of carbon allocation may have contributed to the slow establishment of Caucasian clover, but was not the major factor influencing competitiveness.

The small differences in root/shoot between Caucasian and white clovers supports the contention that the Tt requirement for axillary shoot development was the major factor determining the ability of Caucasian clover to compete in mixed sowings. Both the slower shoot relative growth rate and the higher root/shoot ratio could be explained by late shoot development in Caucasian clover. The appearance and subsequent growth of axillary leaves and branches would depend on the translocation to them of carbon fixed during photosynthesis in the increasing number of leaves (Thomas, 2003).

7.4.6 Implications for Caucasian clover establishment

This study indicates that Caucasian clover seed will establish very slowly when perennial ryegrass and/or white clover are used in the pasture mixture. In some cases, Caucasian clover may even fail to establish. The lack of axillary leaves until 990 °Cd and crown shoots until 1180 °Cd after sowing limits the competitiveness of Caucasian clover seedlings in mixed pastures. It is not only the time of axillary shoot production that is important, but it is the rate of shoot and therefore leaf production after this stage (Figure 7.7) which gives white clover and particularly perennial ryegrass a competitive advantage. The early and subsequently rapid rate of shoot development and shoot relative growth rate of perennial ryegrass confers a substantial competitive advantage over Caucasian clover during the establishment phase when competition for resources, particularly light, is intense. Under these circumstances, even white clover would appear to be too competitive for Caucasian clover. Agronomically, spring sowing of Caucasian clover should be recommended to minimise the interval in days between sowing and exponential leaf production.

The Tt requirement for axillary bud development could be used as a criterion for determining the potential rate of establishment of Caucasian clover for different locations throughout New Zealand. In other words, environments where spring sowing will give rapid seedling development and minimise the time required for axillary shoot development and therefore exponential leaf development.

Table 7.9 lists the theoretic chronological time to axillary bud development for Caucasian clover sown on different dates for a range of sites in New Zealand, based on mean air temperature and assuming soil moisture is non-limiting. In a mild area like Napier on the east coast of the North Island, 67 d are required following a 9 November or 4 February sowing, but this extends to 100 d for 31 March sowing. In contrast, in cooler areas like Riverton, located on the south coast of the South Island, following a 9 November sowing, 91 d are required before axillary bud development is initiated, while Caucasian clover sown on 31 March would require 171 d before initiating axillary bud development. In reality, Caucasian clover may not reach this stage due to competition from associated species or attack by pathogens.

These theoretical assumptions imply that Caucasian clover establishment would be suited to spring sowing alone, particularly in November, to ensure rapid shoot development and minimise the time to axillary bud development. They also show that Caucasian clover will be very slow to establish in cooler locations in Southland for example, or in Cromwell in the South Island high country, even after spring sowing when soil temperatures are above 12 °C and increasing.

Table 7.9 Chronological time in days to initiation of crown shoot development in Caucasian clover at four sites in New Zealand with four sowing dates (actual days calculated from NIWA meteorological data summaries, and Lincoln data from Broadfields meteorological station, using a base temperature of 0 °C).

	Sowing date			
Location	24 September	9 November	4 February	31 March
Lincoln	91	77	81	146
Riverton	104	91	104	171
Cromwell	89	74	82	186
Napier	78	67	67	100

7.5 Conclusions

The series of experiments described in this chapter have provided quantifiable explanations for differences in the rate of establishment observed in the field between Caucasian clover, white clover and perennial ryegrass. Specific conclusions were:

1. The Tt requirement for germination was lower for Caucasian (46 °Cd) and white (40 °Cd) clovers than perennial ryegrass (76 °Cd). However, all three species had similar Tt requirements for emergence (~112 °Cd) and first leaf appearance (~214 °Cd) from a sowing depth of 10 mm.
2. The phyllochron for leaves produced on the primary stem was slower for Caucasian clover (109 °Cd) than white clover (94 °Cd) and perennial ryegrass (101 °Cd).
3. The Tt requirement for the initiation of axillary shoot development was (1180 °Cd for Caucasian clover compared with 440 °Cd for white clover and 373 °Cd for perennial ryegrass. The subsequent rate of shoot and leaf development was also slower in Caucasian clover. This difference provides an explanation for the previously recorded observation of unsuccessful establishment of Caucasian clover when sown with perennial ryegrass.
4. Slow establishment of Caucasian clover was attributed to its slower shoot relative growth rate than white clover and perennial ryegrass from the first to fifth leaves produced in Caucasian clover. However, small differences in root/shoot ratio between species were considered to be a minor contributor for slow establishment of Caucasian clover.
5. These results suggest that successful establishment of Caucasian clover requires spring sowing to minimise the time to axillary shoot development, and without perennial ryegrass or white clover.

Chapter 8

General discussion

8.1 Introduction

The general aim of this thesis was to define the place of Caucasian clover with respect to white clover in temperate pastures (Chapter 1). Constrasts between the two clovers focused on the main environmental constraints of temperature, water status, and soil fertility. Reasons for the poor competitive ability of Caucasian clover seedlings were also sought in relation to commonly sown companion species such as white clover and perennial ryegrass. The objective of this final chapter is to draw results together and compare them with those previously reported in the literature (Chapter 2). General guidelines for the successful inclusion of Caucasian clover in temperate pastures are discussed and topics which require further research are indicated.

8.2 Irrigated or moist conditions

8.2.1 Soil fertility

Caucasian clover was more productive than white clover in irrigated ryegrass pastures under both high (Olsen P 20 μg/ml, sulphate-S 12 μg/g) and low (Olsen P 11 μg/ml, sulphate-S 7 μg/g) soil fertility conditions (Chapter 3). Specifically, mean annual sheep LWG per hectare from CC–RG pastures was 1178 kg on high fertility soils and 1068 kg on low fertility soils over 3 years of rotational grazing. The superior LWG per hectare from CC–RG pastures compared with WC–RG pastures (average ~9%) under both soil fertility conditions was attributed to the ability of Caucasian clover to achieve greater clover content of similar herbage nutritive value relative to white clover. Thus, Caucasian clover has the potential to be more productive than white clover under soil fertility conditions typically found on lowland sheep farms in New Zealand.

The superior performance of Caucasian clover was confirmed when annual yields were measured from the grazed pastures using 28-d regrowth periods under exclosure cages (Chapter 4). The mean annual total DM production for CC–RG pastures in high fertility soil of 17.5 t/ha was 8% more than that for WC–RG pastures. This was associated with a clover DM yield of 4.4 t/ha which was 113% greater than that for WC–RG pastures. In low fertility soil, annual total DM yields were similar for both pasture mixtures (~15.6 t/ha), but clover DM yield for CC–RG of 3.9 t/ha was still 77% greater than that for WC–RG pastures. These results showed that Caucasian clover was superior to white clover under low soil fertility conditions, but the advantage was greatest under high soil fertility conditions where maintenance P and S fertiliser was regularly applied.

Caucasian clover was more productive than white clover under the low soil fertility conditions imposed in this study. This result indicates that Caucasian clover would be more persistent than white clover in environments where less frequent fertiliser P and S inputs are made such as in hill and high country farming areas in New Zealand. A number of studies on Caucasian clover persistence support this conclusion (Section 2.3.3). However, our attempt to achieve a low soil fertility treatment, by withholding fertiliser for more than 10 years, achieved Olsen P (11 μg/ml) and sulphate-S (7 μg/g) levels that were more typical of medium fertility soils in lowland environments. In contrast, low fertility soils in hill and high country environments are often quantified as having an Olsen P of ≤8 μg/ml, a sulphate-S of ≤4 μg/g, and a pH of ≤5.3. Indeed, low fertility in lowland similar to that of hill country would be rare on high value land. This study has not really pushed the boundary of low soil fertility, but has proven that Caucasian clover has an ability to maintain a greater clover content than white clover under a range of medium to adequate soil fertility conditions. What is needed is more data from a wider range of soil fertility levels to challenge the myth that Caucasian clover is only a productive species in infertile soils.

8.2.2 Temperature

Results presented from the grazing experiment (Chapter 4) showed that Caucasian clover was more productive than white clover during spring and summer. Further physiological measurements provided an understanding of reasons for the superior production of Caucasian clover in comparison with white clover (Chapter 5). This was achieved with a detailed study of growth and development responses of the two species to temperature (season) under fully-irrigated conditions.

8.2.2.1 *Spring and summer production*

Caucasian clover had greater leaf photosynthesis rates than white clover at all temperatures tested and the optimum temperature ranges for photosynthesis were similar. This provided a possible explanation for the higher production rates of Caucasian clover observed in mixed pastures. Another factor contributing to rapid spring production of Caucasian clover may be carbon remobilisation from its taproot and rhizomes which could be important for early spring production, but further research is needed to confirm this. This would be apparent in regrowth pastures but not in seedlings. Thus, Caucasian clover has the potential for use in pastures where high spring and summer clover production rates are important.

8.2.2.2 *Autumn production*

Clover production during autumn declined at a faster rate for Caucasian than white clover with decreasing temperatures (Chapter 5). This production difference could be in part explained by the higher base temperature (5 °C) compared with white clover (1 °C), and similar phyllochron (126 °Cd). This means that at the same temperature, the two species have different rates of leaf production and therefore canopy development.

Another reason for low autumn production of Caucasian clover may be that it is partitioning carbon toward root and rhizome production at this time. Seasonal carbon allocation was not measured in this study but it should be an important focus point for future research. Previous research (Section 2.3.4) suggests that seasonal carbon allocation in Caucasian clover parallels that in other deep tap rooted legume species such as lucerne. The taproot and rhizomes of Caucasian clover are the morphological features that confer persistence in Caucasian clover. Thus, management strategies that promote root and rhizome production at this time would be very important for maintaining persistence and productivity of Caucasian clover in temperate pastures. It is likely that Caucasian clover will tolerate frequent grazing during spring and early summer, but less frequent rotational grazing would be advantageous during autumn.

8.3 Dryland conditions (moisture-limiting)

Under dryland conditions, soil water deficit is the overriding factor influencing pasture production. In Chapter 4, results showed that the greatest production advantage for Caucasian clover over white clover was during summer. This difference was attributed to higher leaf photosynthesis rates at any given temperature, but may have also been due to differences in response to water status, given that pastures were not always fully irrigated during summer. An experiment was therefore designed to gain an understanding of the water use efficiency, growth and development of Caucasian clover compared with white clover under dryland conditions.

Dry matter production of Caucasian clover was greater than white clover under dryland conditions due to faster growth rates during periods of both non-limiting and limiting soil water conditions. This difference was attributed to greater water use efficiency and faster leaf photosynthesis rates of Caucasian clover compared with white clover at any given level of soil water. Thus, greater understanding of the physiological reasons for the superior production of Caucasian clover over white clover under dryland conditions was achieved.

Three years after sowing Caucasian clover extracted water to a similar depth to white clover under dryland conditions. This was surprising given that Caucasian clover has a deeper taproot system than white clover and should therefore be able to access water from greater depths. So it was unclear from this study whether or not access to water deeper in the soil profile contributed to Caucasian clover's production advantage under dryland conditions. It should be noted that the water extraction study was conducted on 2 to 3-year-old clover monocultures and it is known that white clover has an effective taproot only in its first 2 years. Furthermore, there may have been some white clover seedling recruitment within the white clover plots during the study so the mean age of the white clover population may have been less than the Caucasian clover population which would have no new seedlings from hard seed in the soil or from reseeding. Over a 6 year period in a summer dry environment at Lincoln, unirrigated Caucasian clover persistence and therefore productivity has been greater than that of white clover (Black and Lucas, 2000). Clearly the deep taproot and rhizome systems of Caucasian clover and shallow nodal root systems of white clover would have contributed to this difference.

Thus, the morphological and physiological differences between Caucasian and white clovers highlighted in this research indicate that Caucasian clover is more suited to summer dry conditions than white clover. These differences suggest that Caucasian clover has the potential to remain productive longer than white clover into the summer when soil water is becoming increasingly unavailable. Both species are unproductive under severe water stress, but Caucasian clover would have greater capacity to recover with autumn rain. Thus, Caucasian clover will have greater persistence than white clover in summer dry areas, but may not be much more productive during droughts.

Caucasian clover is summer active and is more productive in summer moist climates. But the oceanic climate in New Zealand is unpredictable and shallow stony soils and sunny hill slopes will always have dry spells in most years with their limited available soil water. Thus, annual legumes (e.g. subterranean clover) are required in pastures to exploit soil moisture in the cool season and Caucasian clover would exploit years with wet summers. White clover plant populations may recover from surviving stolon fragments and seedling recruitment after rain in summer/autumn periods, but this would be less reliable than Caucasian clover which maintains its growing points below the soil surface.

8.4 Establishment of Caucasian clover

It is clear from this research that Caucasian clover can be more productive than white clover in lowland conditions, and that it is a valuable species in perennial pastures. However, slow establishment has limited the adoption of this species by farmers in contrast to the relative ease of white clover establishment. Thus, a further aim of the research was to develop reliable sowing strategies for successful Caucasian clover establishment.

The results from Chapter 6 demonstrated the sowing strategies required for successful establishment of white clover. Specifically, spring sowing, or autumn sowing when soil temperature is above 14 °C, with low (3–12 kg/ha) seeding rates of perennial ryegrass, resulted in successful establishment of white clover. However, none of the treatments resulted in successful rapid establishment of adequate Caucasian clover populations. Thus, it was necessary to understand the seedling growth and development characteristics of Caucasian clover which could provide an explanation for its slow establishment.

8.4.1 Physiological explanation for slow establishment

The literature has attributed slow establishment of Caucasian clover to its propensity to allocate more carbon to root rather than to shoot development (Section 2.5.3), making it a poor competitor for limited resources, particularly light, during the establishment phase of a pasture (Section 2.5.5). However, results in Chapter 7 showed that slow establishment in Caucasian clover was caused by slow shoot development rather than root production.

The identification of seedling characteristics responsible for the slow establishment of Caucasian clover enabled a greater understanding of its lack of competitive ability during establishment. Carbon partitioning toward below ground root and rhizome production may have contributed in a limited way to its slow establishment, but slow leaf canopy development because of retarded secondary shoot production provided a much more convincing explanation.

8.4.2 Establishment strategies

Now that the physiological reasons for slow establishment in Caucasian clover have been identified, confident recommendations on sowing methods can be made for the establishment of this species. The results presented in Chapters 6 and 7 lead to the conclusion that for establishment of Caucasian clover to be successful it needs to be sown alone in spring. But this is an unusual and generally unacceptable requirement for permanent pasture establishment. This section provides some recommendations for establishment of Caucasian clover under irrigated/moist and dryland conditions.

8.4.2.1 Irrigated or moist conditions

Sowing Caucasian and white clovers in spring and over drilling perennial ryegrass in the following autumn resulted in successful establishment of both species in the grazing experiment (Chapter 3). This method achieved high clover contents by the following spring in experimental situations, but may not be adopted by farmers because of unacceptably low production in the establishment year. Weed control would also be an issue, but if the use of herbicides is already integrated in pasture management on a farm (e.g. annual control of nodding thistles) then reduced production may be accepted. One possible means to overcome low production in the establishment year in moist conditions may be to use a slow establishing grass species such as timothy. Timothy is slow emerging due to its Tt requirement of 200 °Cd and has a slow seedling growth rate (Moot *et al.*, 2000). Competition by timothy for light would be less than from the faster-establishing perennial ryegrass, giving Caucasian clover seedlings greater opportunity to intercept and utilise light for root and rhizome production. Also, like Caucasian clover, timothy herbage has a high nutritive value (Charlton and Stewart, 2000). Thus, sowing these two species together in a pasture mixture provides a useful means of increasing pasture quality in a farming system. Ryegrass can easily be over drilled into the established pasture after 2 to 3 years if the timothy population declines.

8.4.2.2 Dryland conditions

Under sowing with a cover crop presents a potential method for rapid establishment of Caucasian clover under dryland conditions. This approach has been successful for establishment of lucerne under dryland conditions (Wynn-Williams, 1976) and has also been evaluated for the establishment of Caucasian clover for seed and forage production (Seguin *et al.*, 1999; Widdup and Thomas, 2001). Under-sowing Caucasian clover with cover-crops has also been evaluated for dryland pastures at Lincoln University, Canterbury (Moot, pers. comm.). Specifically, spring sowing under a brassica cover crop was the most successful method for establishing Caucasian clover under dryland conditions. An important benefit of this approach is that first defoliation is later than that required for a perennial ryegrass-based pasture thus giving Caucasian clover greater opportunity to reach the secondary shoot development stage and to develop its taproot and rhizome system before moisture becomes limiting during summer. Sowing the cover crop at less than recommended rates provides an intermittent canopy (i.e. LAI of only ~2 cf. ~4 for perennial ryegrass at first grazing) and therefore less competition for light. Alternatively, slow establishing drought tolerant grass species such as cocksfoot or tall fescue enabled Caucasian clover to establish in dryland pastures when sown in early spring (Lucas pers. comm.).

8.4.3 Breeding potential

There has been some research towards selection for rapid establishment in Caucasian clover (DeHaan *et al.*, 2001; Widdup *et al.*, 1998). Selected for rapid emergence and low root/shoot ratios were investigated but the data presented in Chapter 7 showed that seedling emergence is adequate for establishment of Caucasian clover, and that the root/shoot ratio was only a minor factor contributing to its slow establishment. The data showed that the delayed initiation and subsequent slow rate of secondary shoot production were the major factors contributing to slow establishment in Caucasian clover. Rapid initiation of secondary shoot development is a simple characteristic that could be used as a selection criterion in a breeding programme. Selection would be based on those plants that achieved rapid initiation of secondary shoots. Indeed, this feature is probably inadvertently selected for in the Caucasian clover × white clover hybrid programme (Widdup *et al.*, 2003).

8.5 Conclusions

The research presented in this thesis has provided a comprehensive assessment of Caucasian clover performance in relation to white clover in temperate pastures. Specific conclusions were:

1. Caucasian clover was more productive and persistent than white clover in irrigated ryegrass pastures on low fertility soils (Olsen P 11 μg/ml, sulphate-S 7 μg/g), but particularly on high fertility soils (Olsen P 20 μg/ml, sulphate-S 12 μg/g).
2. Caucasian clover had superior DM production rates than white clover during spring and summer, regardless of soil fertility. This was attributed to greater leaf photosynthesis rates in Caucasian clover at any given temperature. Additional research is required to assess the importance of carbon allocation to taproots and rhizomes and the effect of grazing management on this during autumn and the subsequent possible remobilisation in early spring.
3. Caucasian clover was more productive than white clover under summer dryland conditions. This advantage was attributed to Caucasian clover's greater water use efficiency and leaf photosynthesis rates at any given soil moisture level. Additional research is required to quantify the advantages of water extraction from greater depths and protection of underground growing points of rhizomes and crowns of Caucasian clover under summer dry conditions.
4. Caucasian clover is slower to establish than white clover. Slow secondary crown shoot development rather than carbon partitioning to roots provided the major reason for slow establishment of Caucasian clover.
5. Establishment methods for Caucasian clover should be based on its thermal time requirement for first secondary shoot appearance (1180 °Cd). Spring sowing without perennial ryegrass is recommended for rapid Caucasian clover establishment. Additional research and extension to farmers is required to assess the commercial acceptability of sowing strategies which reduce inter-plant competition such as the use of slow establishing grass species (e.g. timothy) under moist conditions, or the use of brassica cover crops or slow establishing grasses (e.g. tall fescue) under dryland conditions. Slow seedling development indicates the need for Caucasian clover to be spring sown.

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Appendix to Chapter 3

Appendix 1 Soil test (0–75 mm) results from May 1998–2001 for Caucasian clover–ryegrass (CC–RG) and white clover–ryegrass (WC–RG) pastures under high (High-F) and low (Low-F) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand.

		pH (H_2O)	Olsen P (μg/ml)	SO_4-S (μg/g)	Ca^{++}	K^+ (meq/100 g)	Mg^{++}	Na^+
May 1998								
High-F	CC–RG	6.1_b	24.0_a	13.0_a	9.1	1.02_a	1.15_{ab}	0.22
	WC–RG	6.1_b	21.0_a	16.0_a	9.1	0.81_b	1.06_b	0.22
Low-F	CC–RG	6.4_a	11.0_b	8.0_b	9.1	1.02_a	1.22_{ab}	0.24
	WC–RG	6.2_b	10.0_b	9.0_b	9.6	1.09_a	1.34_a	0.30
s.e.m.		0.03	0.90	1.20	0.24	0.035	0.041	0.018
May 1999								
High-F	CC–RG	5.9	19.0_a	7.0	7.6	0.77	0.94	0.22
	WC–RG	6.1	18.0_a	7.0	7.6	0.67	0.91	0.23
Low-F	CC–RG	6.1	12.0_b	7.0	7.2	0.77	0.96	0.23
	WC–RG	6.0	14.0_b	6.0	8.1	0.81	0.98	0.21
s.e.m.		0.38	0.70	0.40	0.28	0.049	0.044	0.019
May 2000								
High-F	CC–RG	5.9	18.0_a	7.0_b	5.7	0.60	0.82	0.18
	WC–RG	5.7	14.0_{ab}	11.0_a	6.7	0.60	0.82	0.24
Low-F	CC–RG	5.9	9.0_b	6.0_b	6.2	0.63	0.82	0.20
	WC–RG	5.9	9.0_b	5.0_b	5.7	0.56	0.77	0.20
s.e.m.		0.09	1.60	0.50	0.24	0.049	0.048	0.027
May 2001								
High-F	CC–RG	6.1	23.0_a	20.0_a	6.7	1.09	1.27_{ab}	0.29
	WC–RG	6.1	22.0_a	21.0_a	6.7	1.02	1.30_{ab}	0.35
Low-F	CC–RG	6.2	13.0_b	8.0_b	6.7	1.19	1.37_a	0.33
	WC–RG	6.2	10.0_b	9.0_b	6.2	1.05	1.22_b	0.31
s.e.m.		0.04	1.30	1.20	0.24	0.061	0.023	0.006

Note: Soil samples were analysed using Ministry of Agriculture and Fisheries Quick Test (MAF QT) procedures. Within columns and years, values with the same or no letter subscripts are not significantly different (α=0.05).

Appendix 2 Rotation start and finish dates, rotation duration, and mean grazing duration per plot from 11 September 1998 to 1 May 2001 in block H17 at Lincoln University, Canterbury, New Zealand.

Year	Rotation	Start date	Finish date	Rotation duration (d)	Grazing duration (d/plot)
1998/99	1	11 Sep.	28 Sep.	17	4.3
	2	28 Sep.	14 Oct.	16	4.0
	3	14 Oct.	29 Oct.	15	3.8
	4	29 Oct.	16 Nov.	18	4.5
	5	16 Nov.	11 Dec.	25	6.3
	6	11 Dec.	18 Dec.	7	1.8
	7†	23 Dec.	11 Jan.	19	4.8
	8	5 Feb.	2 Mar.	25	6.3
	9	3 Mar.	31 Mar.	28	7.0
	10	1 Apr.	28 Apr.	27	6.8
	11	10 May	10 Jun.	31	7.8
1999/00	1	14 Sep.	28 Sep.	14	3.5
	2	28 Sep.	22 Oct.	24	6.0
	3	3 Nov.	18 Nov.	15	3.8
	4	18 Nov.	3 Dec.	15	3.8
	5	14 Dec.	5 Jan.	22	5.5
	6†	5 Jan.	31 Jan.	26	6.5
	7	11 Feb.	13 Mar.	31	7.8
	8	21 Mar.	19 Apr.	29	7.3
	9	27 Apr.	23 May	26	6.5
2000/01	1	14 Sep.	28 Sep.	14	3.5
	2	29 Sep.	16 Oct.	17	4.3
	3	16 Oct.	5 Nov.	20	5.0
	4	5 Nov.	24 Nov.	19	4.8
	5	24 Nov.	13 Dec.	19	4.8
	6†	13 Dec.	4 Jan.	22	5.5
	7†	4 Jan.	29 Jan.	25	6.3
	8	12 Feb.	13 Mar.	29	7.3
	9	13 Mar.	11 Apr.	29	7.3
	10†	11 Apr.	1 May	20	5.0

Note: † Liveweight gain was not measured in summer and from 11 April 2001 to 1 May 2001 as described in Section 3.2.10.2.

Appendix 3 Mean crude protein (CP), digestible organic matter in the dry matter (DOMD), and metabolisable energy (ME) concentrations in clover green leaf herbage in pre-grazing herbage mass in spring, summer, and autumn grazing periods for Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (High-F) and low (Low-F) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand.

Year		CP (%)			DOMD (%)			ME (MJ/kg DM)		
		Spr.	Sum.	Aut.	Spr.	Sum.	Aut.	Spr.	Sum.	Aut.
1998/99										
High-F	CC	30.1_{ab}	29.1	30.7_{a}	77.2_{b}	76.6	78.4	12.4_{b}	12.3	12.5
	WC	29.6_{ab}	27.7	27.6_{b}	78.6_{a}	76.8	79.6	12.6_{a}	12.3	12.7
Low-F	CC	30.4_{a}	30.0	29.3_{a}	77.6_{b}	76.2	78.4	12.4_{b}	12.2	12.5
	WC	28.7_{b}	29.3	27.7_{b}	77.8_{ab}	76.5	79.6	12.4_{b}	12.2	12.7
s.e.m.		0.27	0.78	0.28	0.23	0.66	–†	0.05	0.10	–
1999/00										
High-F	CC	29.1	30.4_{a}	30.7	78.3	80.1	78.6	12.5	12.8	12.6
	WC	28.1	28.6_{b}	29.9	78.1	79.4	77.5	12.5	12.7	12.4
Low-F	CC	30.3	31.0_{a}	29.9	78.5	79.3	78.5	12.6	12.7	12.6
	WC	29.7	28.2_{b}	29.5	78.1	81.2	77.8	12.5	13.0	12.4
s.e.m.		0.78	0.17	0.50	0.53	0.77	1.08	0.06	0.13	0.17
2000/01										
High-F	CC	31.5_{a}	30.2_{a}	28.9	78.0	76.7	77.7	12.5	12.3	12.4
	WC	27.8_{ab}	27.6_{b}	30.7	77.8	76.9	77.4	12.4	12.3	12.4
Low-F	CC	30.4_{a}	30.2_{a}	30.7	77.7	76.6	77.7	12.4	12.3	12.4
	WC	25.6_{b}	27.6_{b}	29.9	78.0	77.3	77.4	12.5	12.4	12.4
s.e.m.		1.19	0.57	0.89	0.43	0.21	0.29	0.06	0.05	0.06

Note: Details of soil fertility treatments are given in Section 3.2.3. Details of spring, summer and autumn grazing periods are given in Section 3.2.6. Within columns, values with the same or no letter subscripts are not significantly different (α=0.05). †Values are the same for all replicates so s.e.m. could not be calculated.

Appendix 4 Mean crude protein (CP), digestible organic matter in the dry matter (DOMD), and metabolisable energy (ME) concentrations in ryegrass green leaf herbage in pre-grazing herbage mass in spring, summer and autumn grazing periods for Caucasian clover–ryegrass (CC) and white clover–ryegrass (WC) pastures under high (High-F) and low (Low-F) soil fertility conditions in block H17 at Lincoln University, Canterbury, New Zealand.

Year		CP (%)			DOMD (%)			ME (MJ/kg DM)		
		Spr.	Sum.	Aut.	Spr.	Sum.	Aut.	Spr.	Sum.	Aut.
1998/99										
High-F	CC	23.3	23.2	24.5	73.6	72.1	71.5	11.8	11.5	11.4
	WC	22.2	22.3	23.9	72.3	72.1	70.7	11.6	11.5	11.3
Low-F	CC	22.4	24.1	24.1	70.9	72.2	71.5	11.4	11.6	11.4
	WC	21.9	24.0	23.8	72.5	73.1	70.7	11.6	11.7	11.3
s.e.m.		0.66	0.50	0.39	0.86	0.84	–[†]	0.13	0.14	–
1999/00										
High-F	CC	22.1	22.6	24.1	73.0	69.2	71.1	11.7	11.1	11.4
	WC	21.6	21.3	23.1	72.4	70.7	68.7	11.6	11.3	11.0
Low-F	CC	22.9	21.1	22.8	72.0	70.8	70.4	11.5	11.3	11.3
	WC	21.7	21.2	22.8	72.8	71.2	69.9	11.6	11.4	11.2
s.e.m.		0.44	0.59	0.35	0.44	0.41	0.79	0.05	0.08	0.13
2000/01										
High-F	CC	22.1	21.9	23.0	75.4	71.9	72.7	12.1	11.5	11.6
	WC	22.0	20.9	23.0	74.0	70.5	71.8	11.8	11.3	11.5
Low-F	CC	21.2	21.5	22.9	73.3	71.0	72.9	11.7	11.4	11.7
	WC	20.8	21.1	22.0	73.7	72.7	72.3	11.8	11.6	11.6
s.e.m.		0.38	0.54	0.38	0.57	0.60	0.40	0.09	0.12	0.06

Note: Details of soil fertility treatments are given in Section 3.2.3. Details of spring, summer and autumn grazing periods are given in Section 3.2.6. Within columns, values with the same or no letter subscripts are not significantly different (α=0.05). [†]Values are the same for all replicates so s.e.m. could not be calculated.

Appendix 5 Macro nutrient concentrations in clover leaves plus petioles for Caucasian (CC) and white (WC) clovers under high (High-F) and low (Low-F) soil fertility conditions in December 1998, December 1999 and November 2001 in block H17 at Lincoln University, Canterbury, New Zealand.

		Macro nutrient concentrations (%)						
		N	P	S	Mg	Ca	Na	K
December 1998								
High-F	CC	3.89	0.35	0.22	0.30_a	1.40_a	0.02_c	2.72_b
	WC	3.69	0.32	0.20	0.26_b	1.22_a	0.35_a	2.47_c
Low-F	CC	3.75	0.32	0.22	0.28_{ab}	1.27_a	0.02_c	2.89_b
	WC	3.74	0.33	0.22	0.23_c	1.04_b	0.21_b	3.07_a
s.e.m.		0.095	0.009	0.010	0.008	0.059	0.026	0.052
December 1999								
High-F	CC	4.16	0.36	0.23	0.30	1.36	0.01	2.85
	WC	3.79	0.33	0.21	0.28	1.31	0.23	2.82
Low-F	CC	4.14	0.34	0.22	0.29	1.33	0.01	2.94
	WC	3.73	0.35	0.23	0.34	1.28	0.23	3.11
s.e.m.		–[†]	–	–	–	–	–	–
November 2001								
High-F	CC	4.53_a	0.34	0.25	0.28	1.36_a	0.01_b	2.73
	WC	4.27_a	0.31	0.24	0.26	1.22_b	0.40_a	2.57
Low-F	CC	4.50_a	0.32	0.24	0.27	1.36_a	0.01_b	2.85
	WC	3.94_b	0.31	0.22	0.25	1.15_b	0.37_a	2.80
s.e.m.		0.072	0.007	0.010	0.011	0.031	0.021	0.185

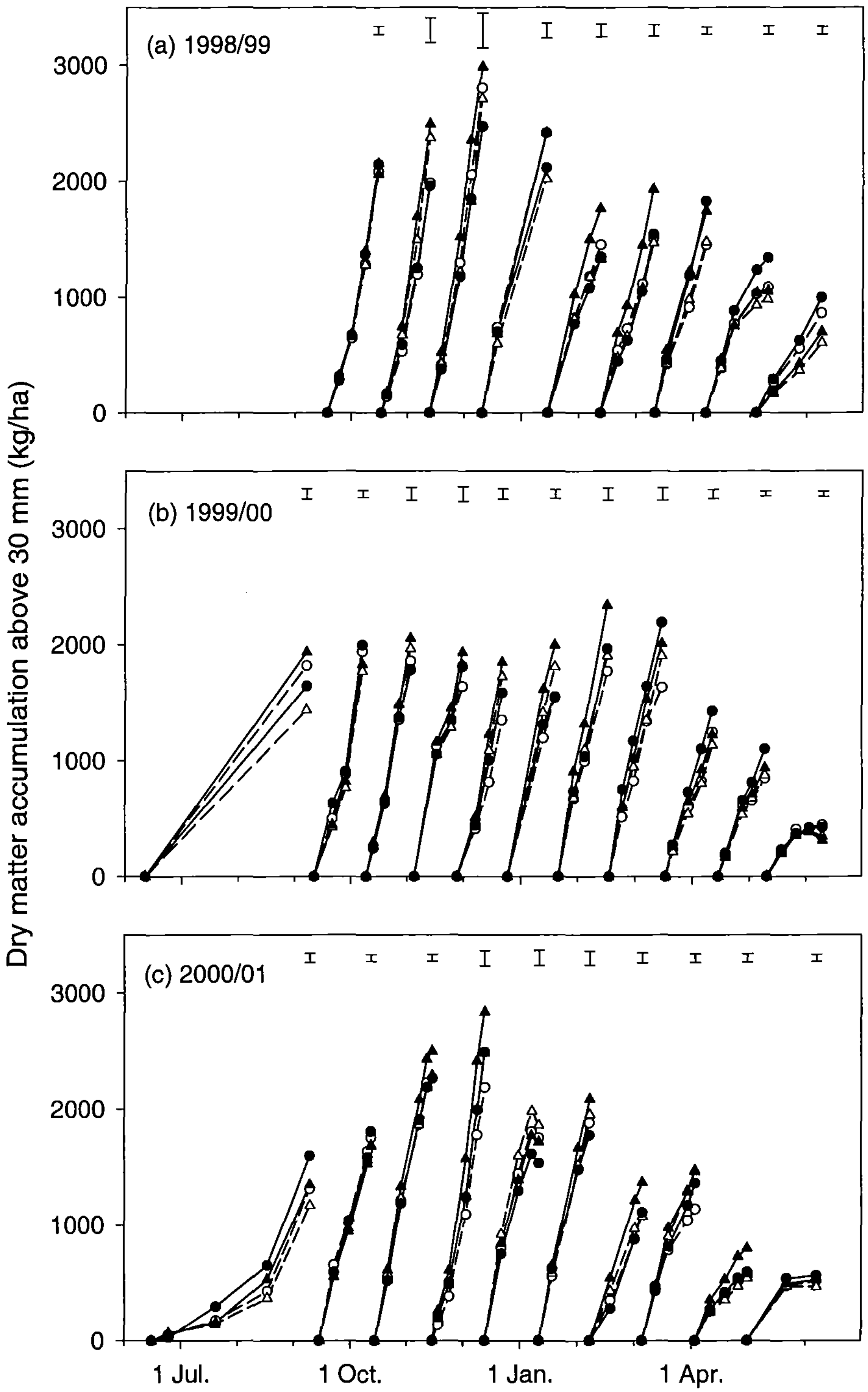
Note: Details of soil fertility treatments are given in Section 3.2.3. Within columns, values with the same or no letter subscripts are not significantly different (α=0.05). [†]Samples were only analysed from one replicate s.e.m. could not be calculated.

Appendix 6 Micro nutrient concentrations in clover leaves plus petioles for Caucasian (CC) and white (WC) clovers under high (High-F) and low (Low-F) soil fertility conditions in December 1998 in block H17 at Lincoln University, Canterbury, New Zealand.

		Micro nutrient concentrations (ppm)					
		Mn	Zn	Cu	Fe	B	Mo
High-F	CC	24	25_{ab}	5	82	25	0.25_{b}
	WC	31	21_{bc}	5	107	24	0.33_{b}
Low-F	CC	32	26_{a}	6	96	24	0.29_{b}
	WC	31	22_{b}	6	102	24	0.48_{a}
s.e.m.		1.8	1.1	0.4	6.2	1.1	0.04

Note: Details of soil fertility treatments are given in Section 3.2.3. Within columns, values with the same or no letter subscripts are not significantly different (α=0.05).

Appendix to Chapter 4



Appendix 7 Dry matter accumulation (above 30 mm) for Caucasian clover–ryegrass (▲,Δ) and white clover–ryegrass (●,○) pastures under high (▲,●) and low (Δ,○) soil fertility conditions from 1 July 1998 to 30 June 2001 in block H17 at Lincoln University, Canterbury, New Zealand. Bars represent standard error of the mean for final yields.

Appendix to Chapter 5

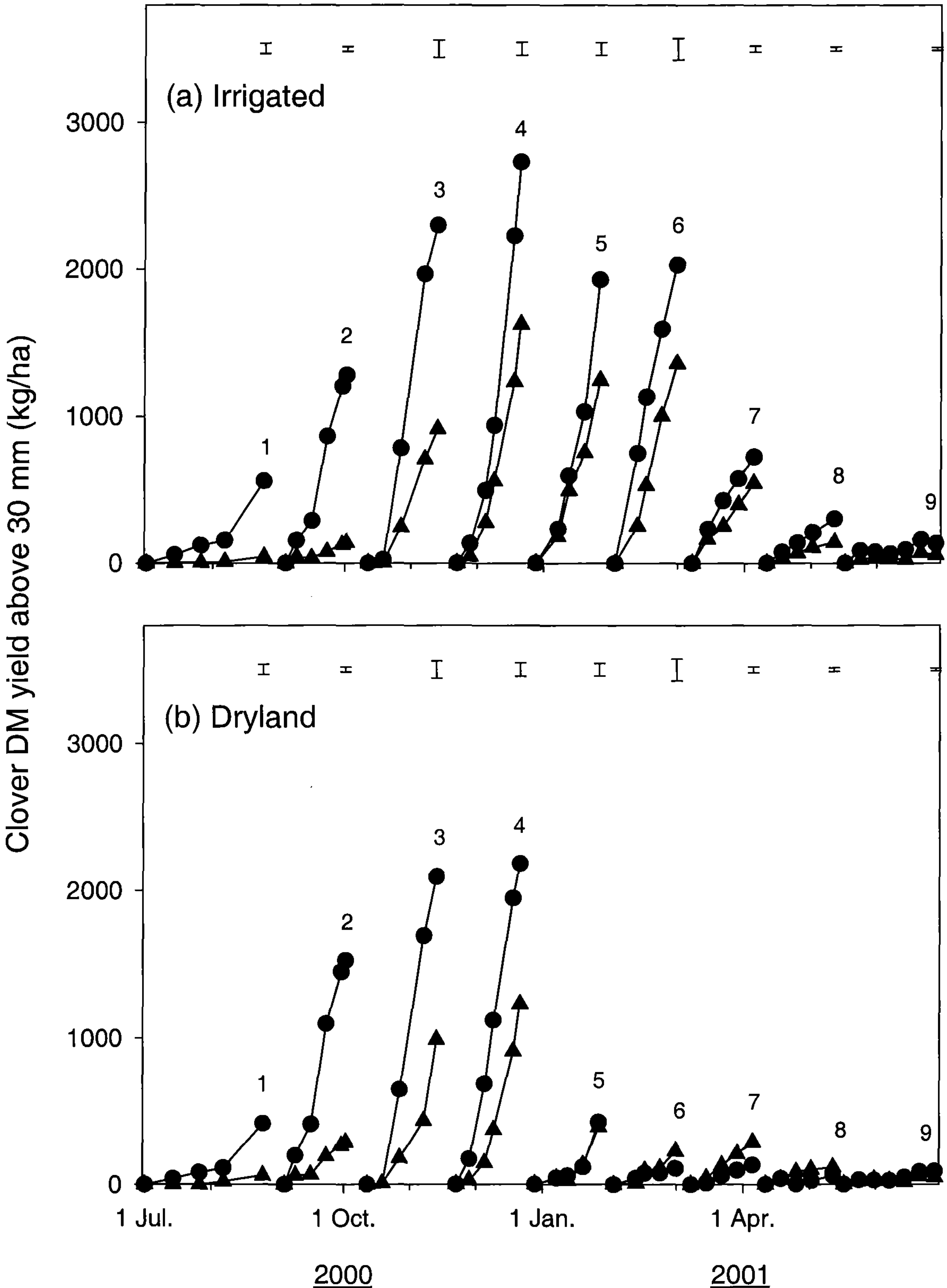
Appendix 8 Amount and timing of irrigation water applied for two years from 1 July 2000 to 30 June 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand.

Year	Regrewth period	Appication date	Irrigation (mm)
2000/01	4	21–23 Nov.	50
2 years old	4	13–18 Dec.	90
	5	8–16 Jan.	72
	6	3–14 Feb.	95
	7	9–17 Mar.	95
	8	10–20 Apr.	105
Total			507
2001/02	1	22 Aug.–20 Sep.	48
3 years old	3	19–27 Nov.	65
	4	20–23 Dec.	80
	6	15–24 Mar.	106
Total			299
2001/02 Dryland*		22 Aug.–20 Sep.	166

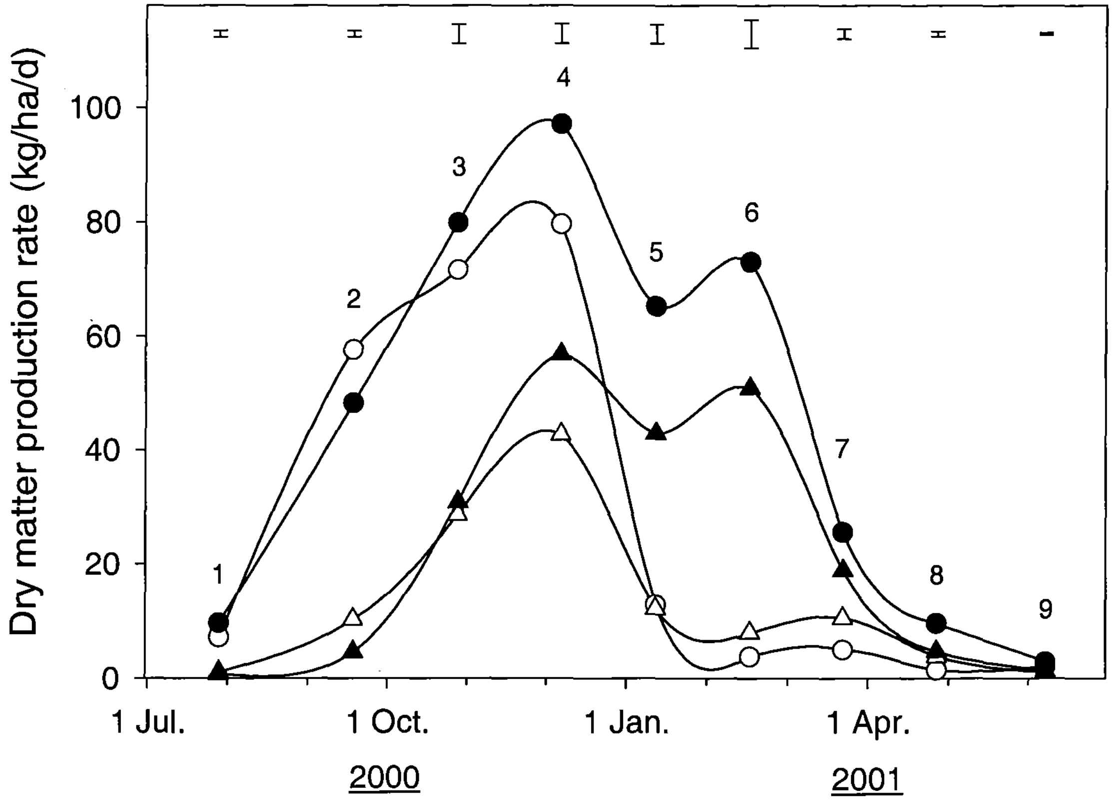
Note: *Irrigation water was applied to the dryland treatment because rainfall had not recharged soil moisture completely in winter.

Appendix 9 Regrowth period start and finish dates, and regrowth and grazing durations from 1 July 2000 to 2 July 2002 in Iversen 10 at Lincoln University, Canterbury, New Zealand.

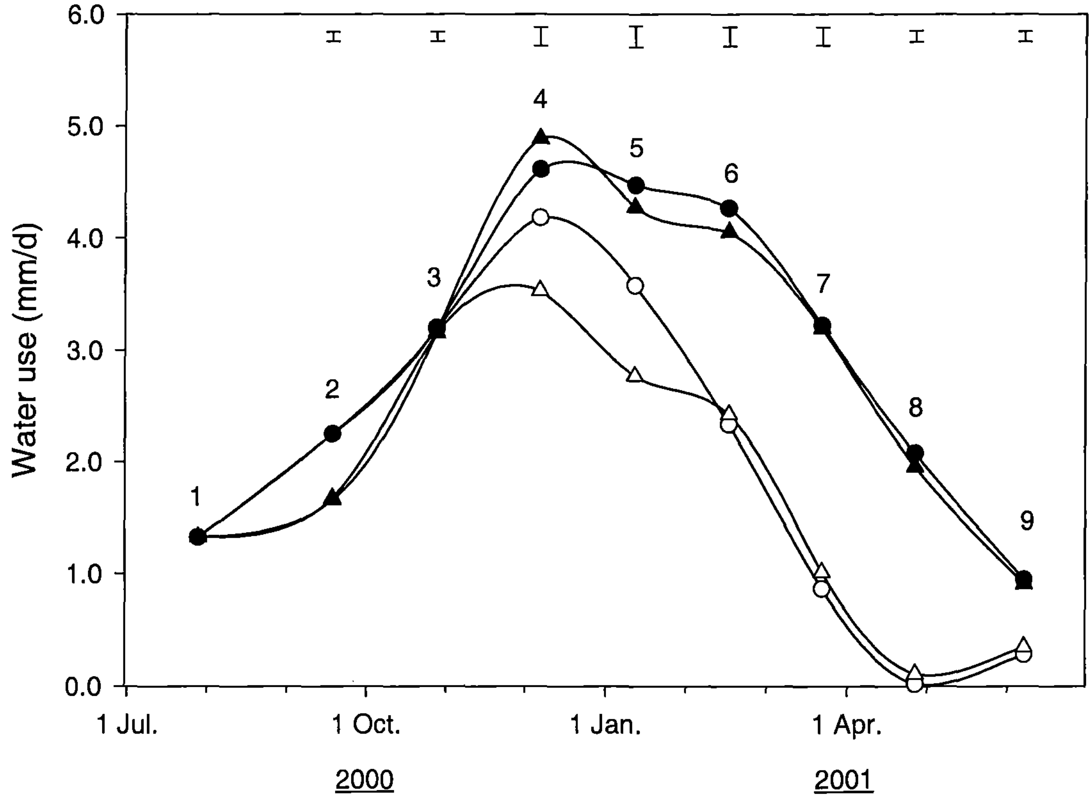
Season	Regrowth period	Start date	Finish date	Regrowth duration (d)	Grazing duration (d)
2000/01	1	1 Jul.	24 Aug.	54	10
2 years old	2	3 Sep.	1 Oct.	28	10
	3	11 Oct.	12 Nov.	32	9
	4	21 Nov.	20 Dec.	29	7
	5	27 Dec.	25 Jan.	29	7
	6	1 Feb.	1 Mar.	28	7
	7	8 Mar.	5 Apr.	28	6
	8	11 Apr.	12 May	31	5
	9	17 May	28 Jun.	42	14
2001/02	1	12 Jul.	20 Sep.	70	6
3 years old	2	26 Sep.	2 Nov.	37	10
	3	12 Nov.	16 Dec.	34	7
	4	23 Dec.	24 Jan.	32	11
	5	4 Feb.	10 Mar.	34	4
	6	14 Mar.	23 Apr.	40	7
	7	30 Apr.	25 Jun.	56	7



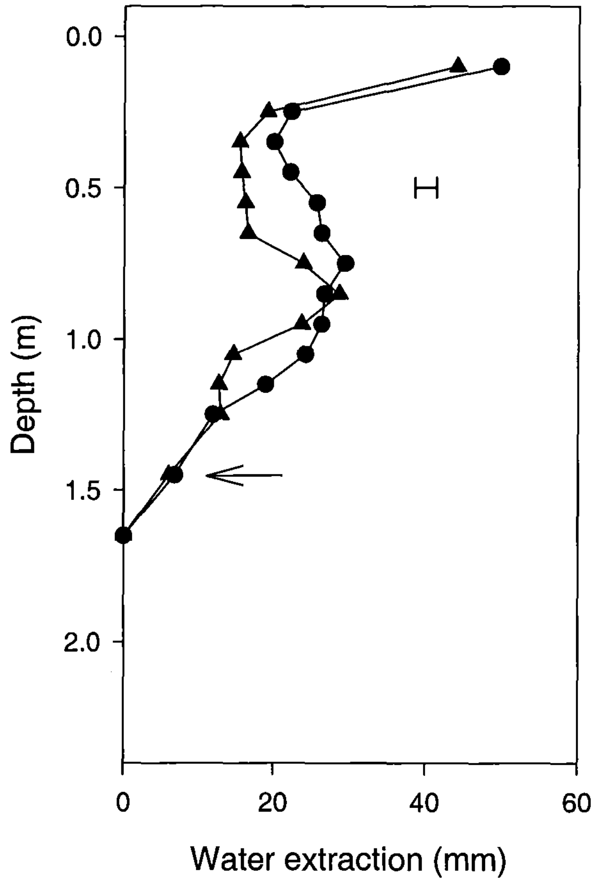
Appendix 10 Clover dry matter (DM) yield accumulation (above 30 mm) for 2-year-old Caucasian (▲) and white (•) clovers under irrigated and dryland conditions from 1 July 2000 to 30 June 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean for final yields. Each regrowth period is numbered 1–9.



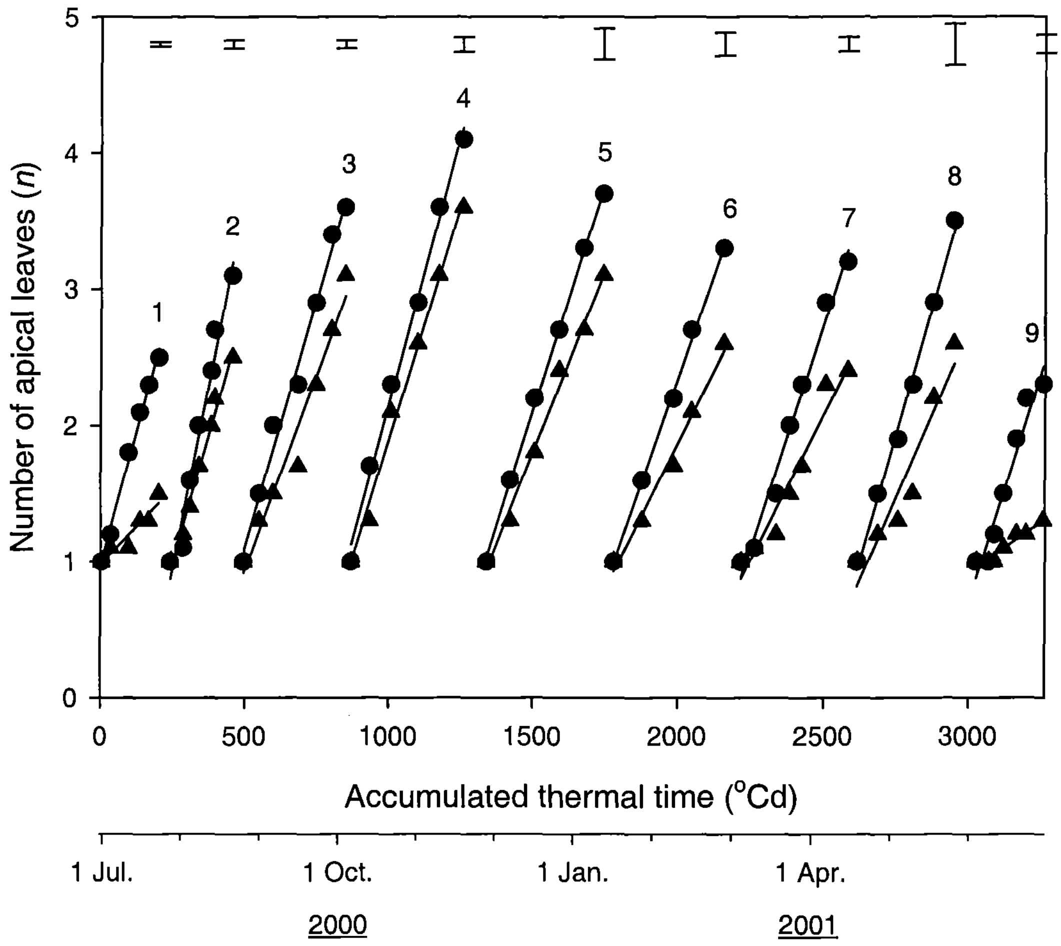
Appendix 11 Dry matter production rate for 2-year-old Caucasian (▲,Δ) and white (●,○) clovers under irrigated (▲,●) and dryland (Δ,○) conditions from 1 July 2000 to 30 June 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean. Each regrowth period is numbered 1–9.



Appendix 12 Seasonal water use for 2-year-old Caucasian (▲,Δ) and white (●,○) clovers under irrigated (▲,●) and dryland (Δ,○) conditions from 1 July 2000 to 30 June 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars indicate standard error of the mean. Each regrowth period is numbered 1–9.

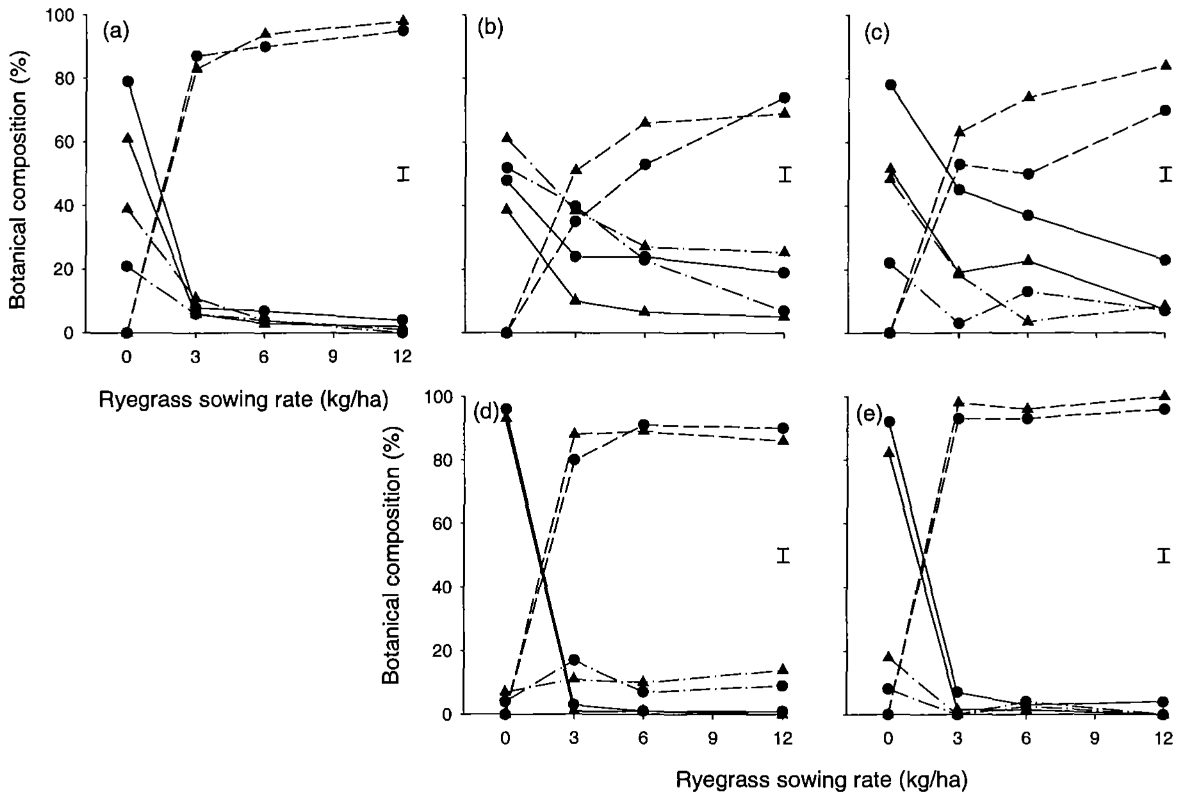


Appendix 13 Water extraction patterns for 2-year-old Caucasian (▲) and white (•) clovers under dryland conditions from 12 September 2000 to 25 April 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bar represents the standard error of the mean. Arrow indicates the maximum water extraction depth for both species.

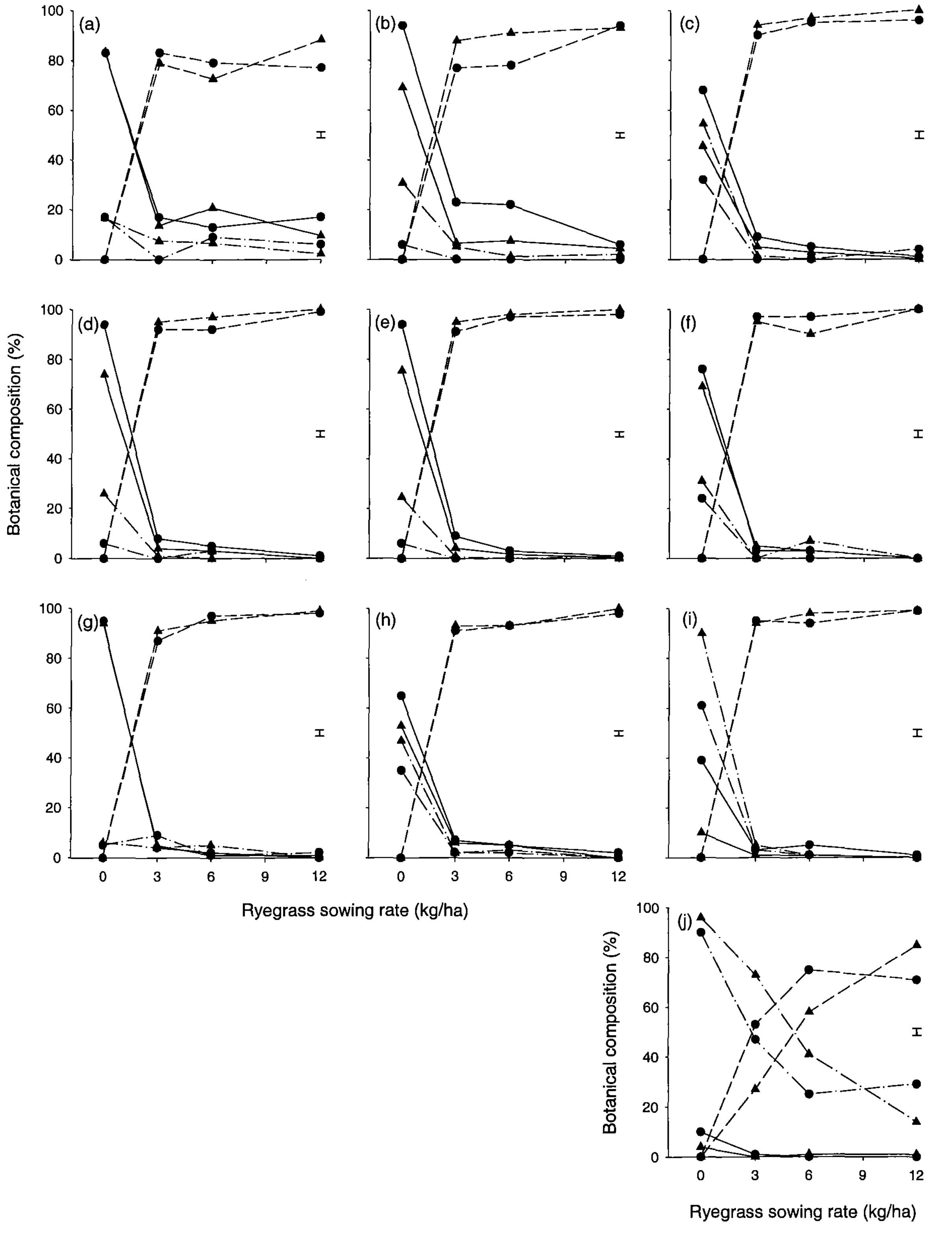


Appendix 14 Leaf appearance rate for apical leaves of Caucasian (▲) and white (•) clovers as a function of accumulated thermal time under dryland conditions from 1 July 2000 to 30 June 2001 in Iversen 10 at Lincoln University, Canterbury, New Zealand. Bars represent the standard error of the mean for final number of leaves. Each regrowth period is numbered 1–9.

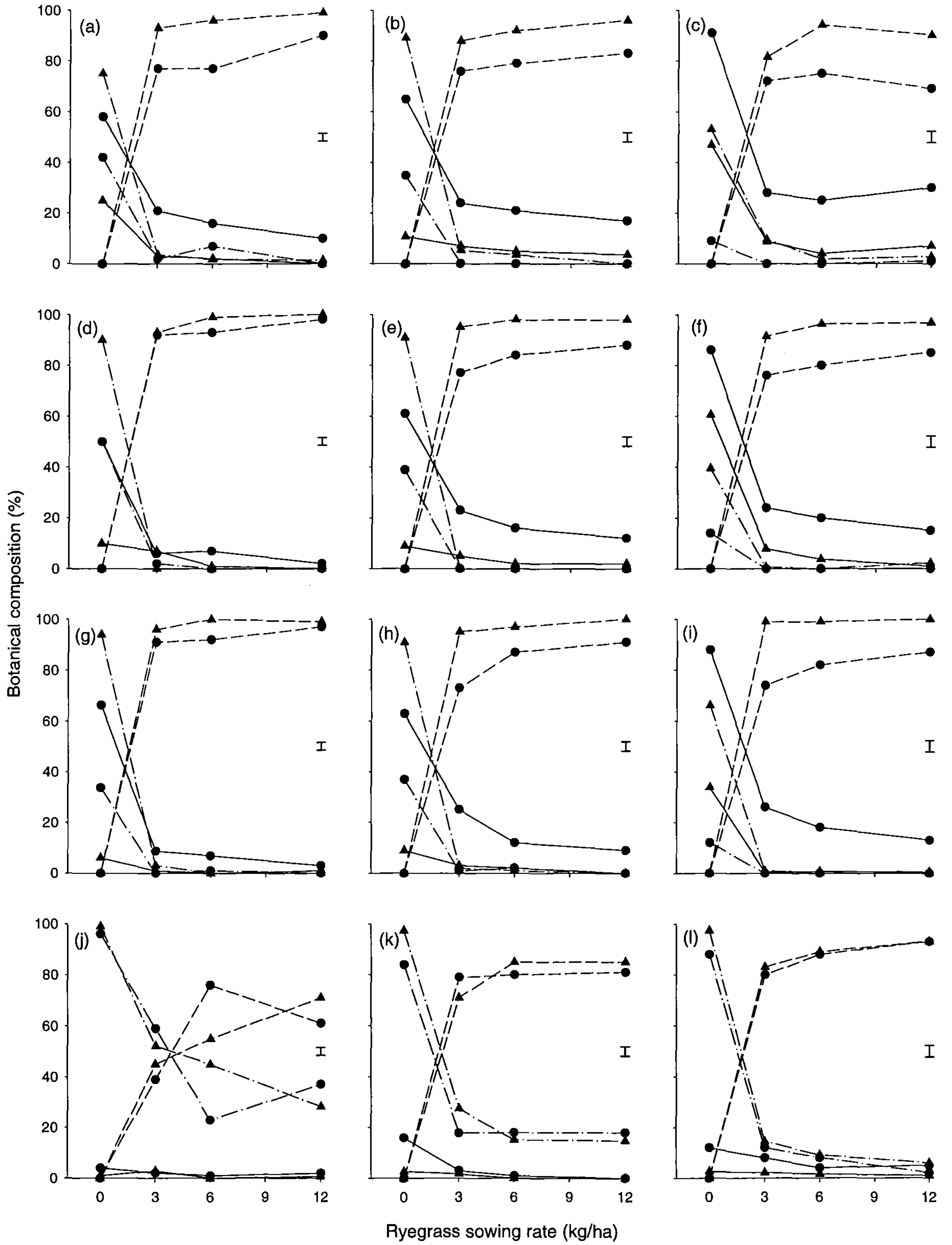
Appendix to Chapter 6



Appendix 15 Percentage of ryegrass (----), clover (——) and weeds (–·–·) on 27 November 1999 (a), 12 January 2000 (b,d) and 18 February 2000 (c,e) in Caucasian (▲) and white (●) clover pastures sown on 24 September 1999 (a,b,c) and 9 November 1999 (d,e) with four perennial ryegrass sowing rates. Bars represent the maximum standard error of means.



Appendix 16 Percentage of ryegrass (----), clover (——) and weeds (–·–) on 1 April 2000 (a,d,g), 10 May 2000 (b,e,h) and 30 June 2000 (c,f,i,j) in Caucasian (▲) and white (●) clover pastures sown on 24 September 1999 (a,b,c), 9 November 1999 (d,e,f), 4 February 2000 (g,h,i) and 31 March 2000 (j) with four perennial ryegrass sowing rates. Bars represent the maximum standard error of means.



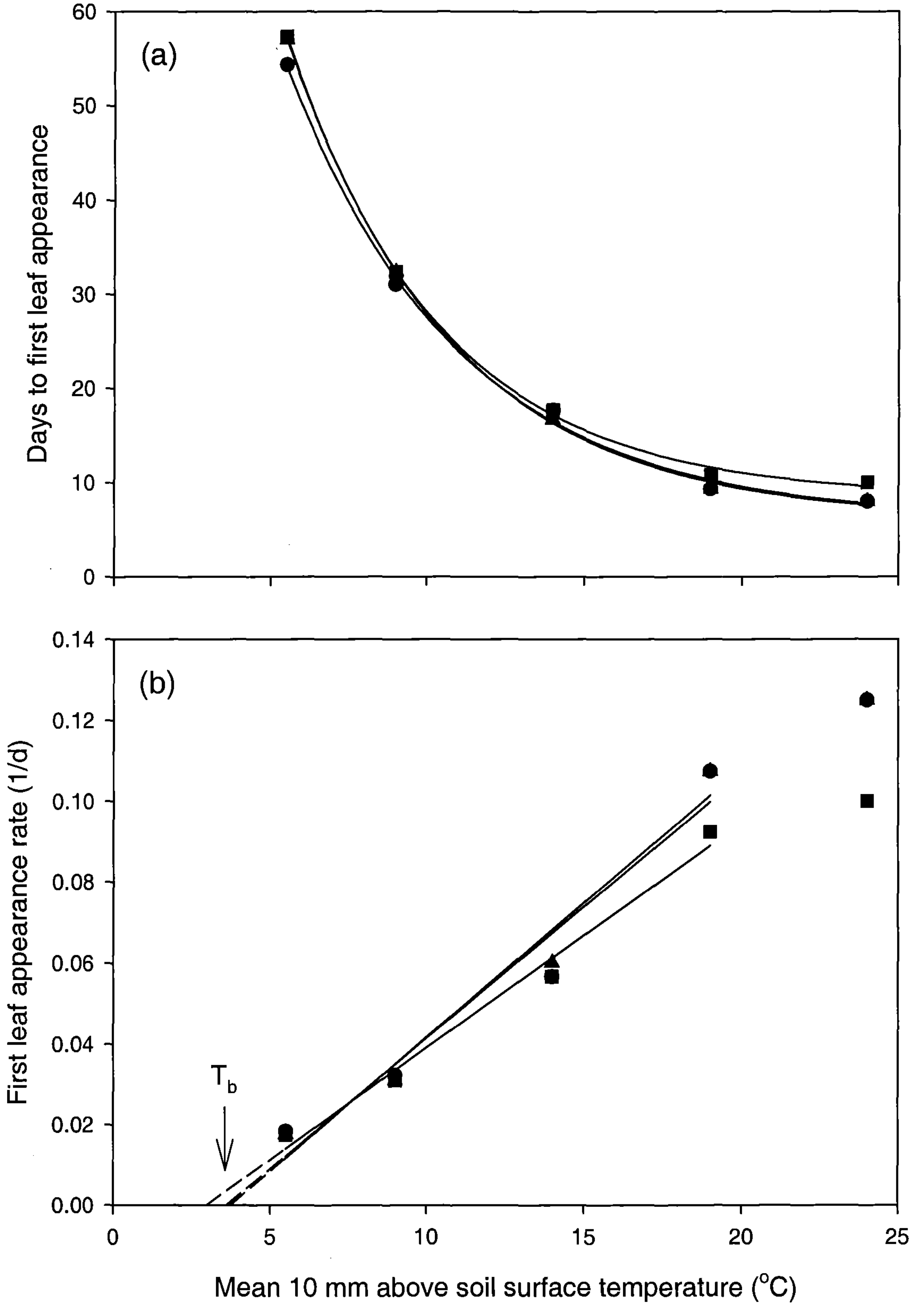
Appendix 17 Percentage of ryegrass (----), clover (——) and weeds (-·-) on 22 August 2000 (a,d,g,j), 3 October 2000 (b,e,h,k) and 6 November 2000 (c,f,i,l) in Caucasian (▲) and white (●) clover pastures sown on 24 September 1999 (a,b,c), 9 November 1999 (d,e,f), 4 February 2000 (g,h,i) and 31 March 2000 (j,k,l) with four perennial ryegrass sowing rates. Bars represent the maximum standard error of means.

Appendix 18 Total dry matter production (kg DM/ha) at each harvest date up to 6 November 2000 from Caucasian (CC) and white (WC) clover pastures sown on four sowing dates with different ryegrass sowing rates.

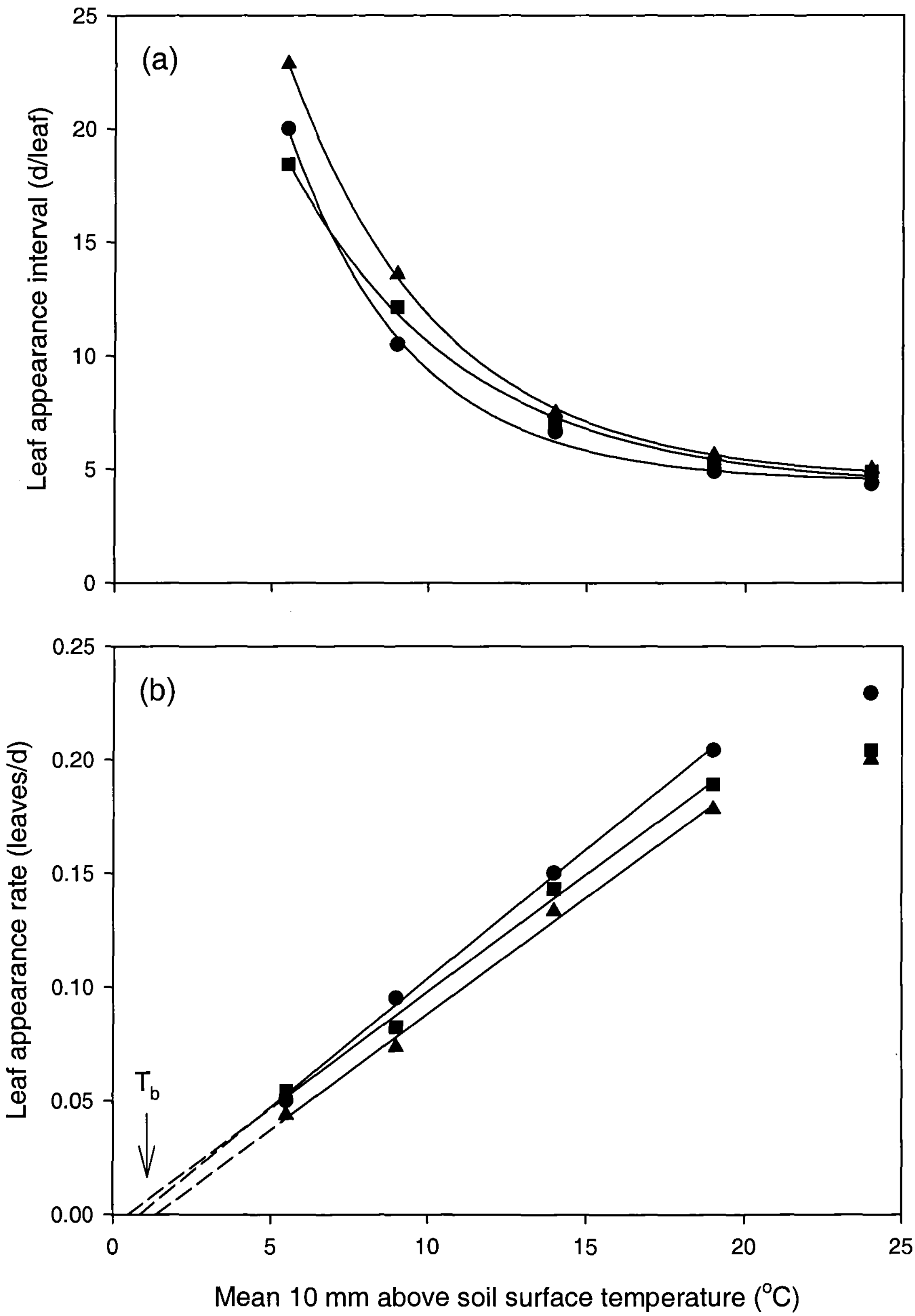
	Harvest date									
	27 Nov.	12 Jan.	18 Feb.	1 Apr.	10 May	30 Jun.	22 Aug.	3 Oct.	6 Nov.	Total
Clover (C)										
CC	890_b	1740_a	2000_a	1230_a	1070_b	1170_a	1270_b	1460_b	1780_b	9500_b
WC	940_a	1800_a	2070_a	1240_a	1210_a	1230_a	1370_a	1650_a	2120_a	10380_a
s.e.m.	15	43	82	32	30	27	30	35	44	114
P_C	0.041	0.303	0.562	0.761	0.003	0.081	0.018	<0.001	<0.001	<0.001
Sowing rate (R)										
0 kg/ha	190_d	590_d	780_c	470_c	460_b	590_d	1120_c	1450_b	1630_b	6220_c
3	840_c	1340_c	2150_b	1300_b	1340_a	1200_c	1330_b	1590_{ab}	2100_a	10450_b
6	1220_b	2110_b	2460_b	1540_a	1380_a	1440_b	1470_a	1700_a	2140_a	11520_a
12	1420_a	2450_a	2760_a	1620_a	1380_a	1570_a	1360_{ab}	1480_b	1960_a	11580_a
s.e.m.	21	61	116	46	43	38	42	49	62	161
P_R	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001
Sowing date (D)										
24 Sep.	920	2150_a	2000_a	1310_a	1270_a	1330_{ab}	1430_a	1770_a	2210_a	14380_a
9 Nov.	–	1390_b	2080_a	1520_a	1310_a	1450_a	1290_a	1500_a	1670_a	12200_b
4 Feb.	–	–	–	870_b	840_a	1200_b	1260_a	1590_a	1940_a	7710_c
31 Mar.	–	–	–	–	–	820_c	1300_a	1350_a	2010_a	5480_d
s.e.m.		22	197	80	91	45	51	98	99	246
P_D		0.026	0.832	0.055	0.111	0.007	0.278	0.180	0.105	<0.001
Interactions	none	none	none	DxR	DxR 0.036	none	DxR	DxR	DxR	DxR
(P<0.05)				<0.001	CxR 0.022		<0.001	<0.001	<0.001	<0.001
				CxR 0.020						DxC 0.040
										CxR 0.002

Note: Within columns and main effects, values with the same letter subscript are not significantly different (α=0.05) according to Fisher's protected least significant difference test.

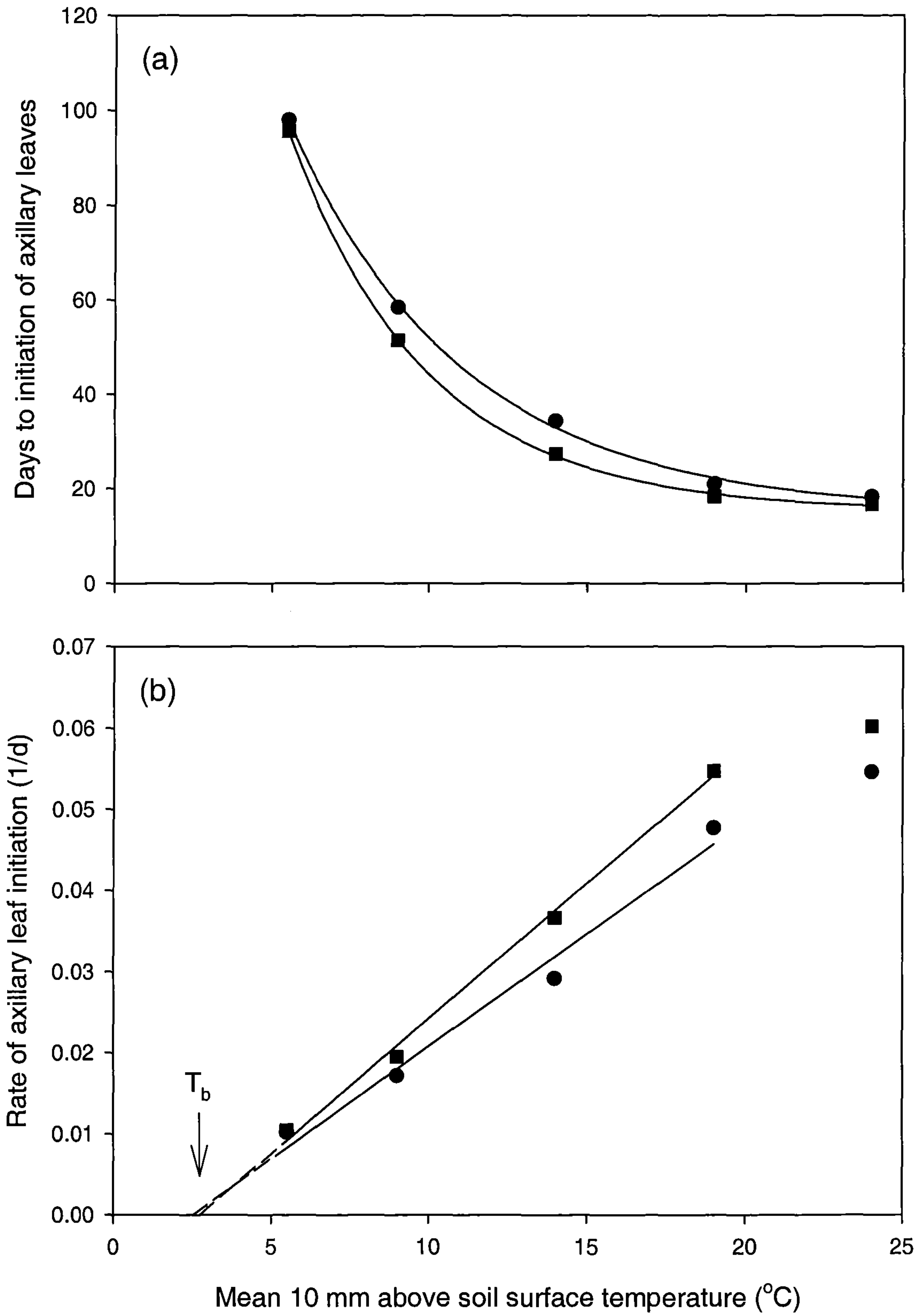
Appendix to Chapter 7



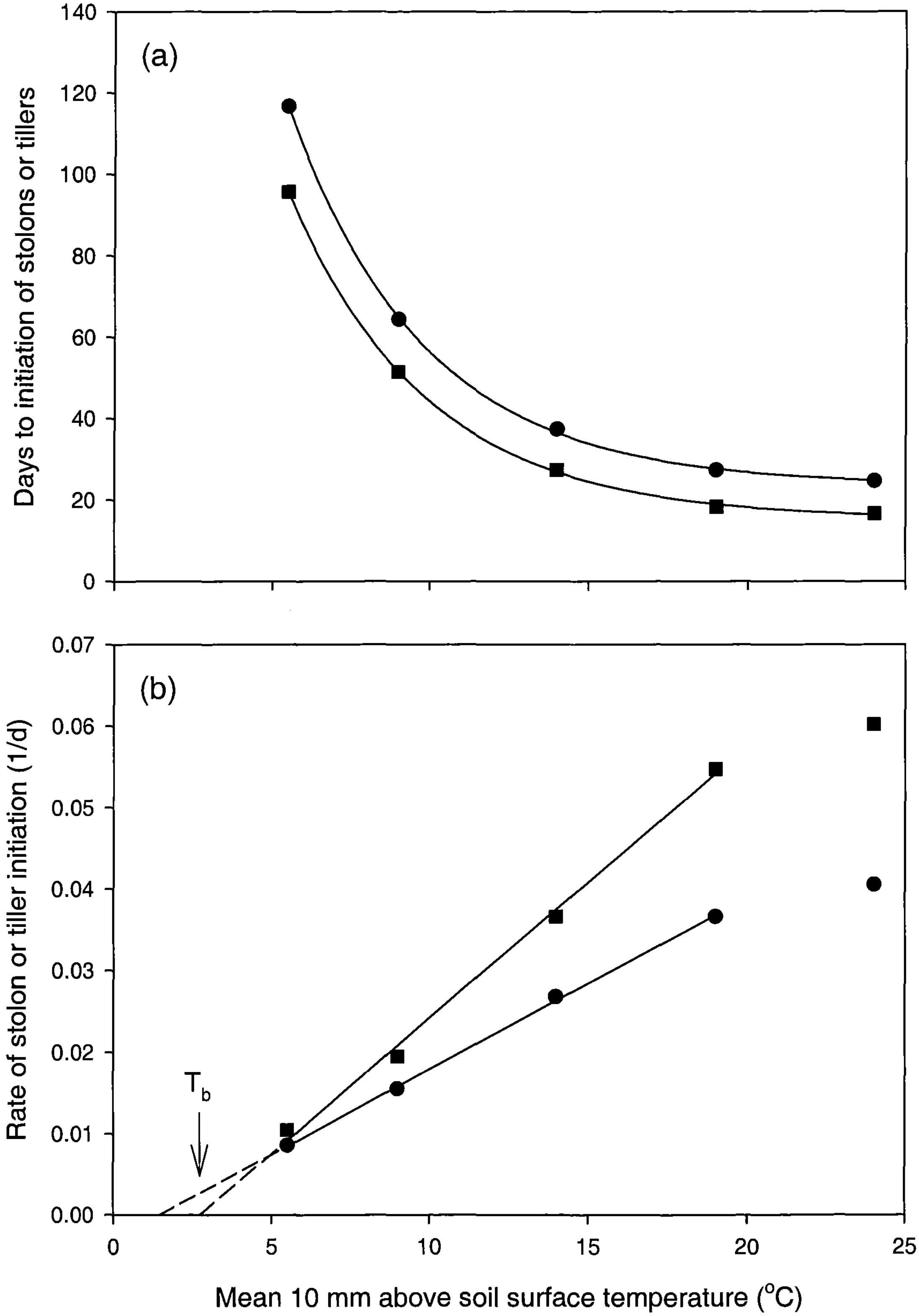
Appendix 19 Number of days to first (unifoliate in clovers) leaf appearance (a) and first leaf appearance rate (b) of Caucasian clover (▲), white clover (●) and perennial ryegrass (■) at different temperatures.



Appendix 20 Leaf appearance interval (a) and leaf appearance rate (b) on the primary stem of Caucasian clover (▲), white clover (●) and perennial ryegrass (■) at different temperatures. Calculated from the first (unifoliate) leaf to the fifth leaf on the primary stem.



Appendix 21 Number of days to initiation of axillary leaves (a) and rate of axillary leaf initiation (b) of white clover (●) and perennial ryegrass (■) at different temperatures.



Appendix 22 Number of days to initiation of stolons or tillers (a) and rate of stolon or tiller initiation (b) of white clover (●) and perennial ryegrass (■) at different temperatures.

Plates

Plate 1 View in October 2000 of part of the Caucasian clover versus white clover grazing experiment in block H17 at Lincoln University, Canterbury, New Zealand. Note the eight treatment flocks of 'Coopworth' ewe hoggets, and larger groups of spare animals in the background that grazed off the experimental site. In the foreground, note the cage area of pasture and the previous cage area (marked with a white peg) that were part of the study described in Chapter 4.

Plate 2 'Grasslands Demand' white clover/'Grasslands Ruanui' perennial ryegrass pasture in October 1997 in block H17 at Lincoln University, Canterbury, New Zealand.

Plate 3 'Endura' Caucasian clover/'Grasslands Ruanui' perennial ryegrass pasture in October 1997 in block H17 at Lincoln University, Canterbury, New Zealand.

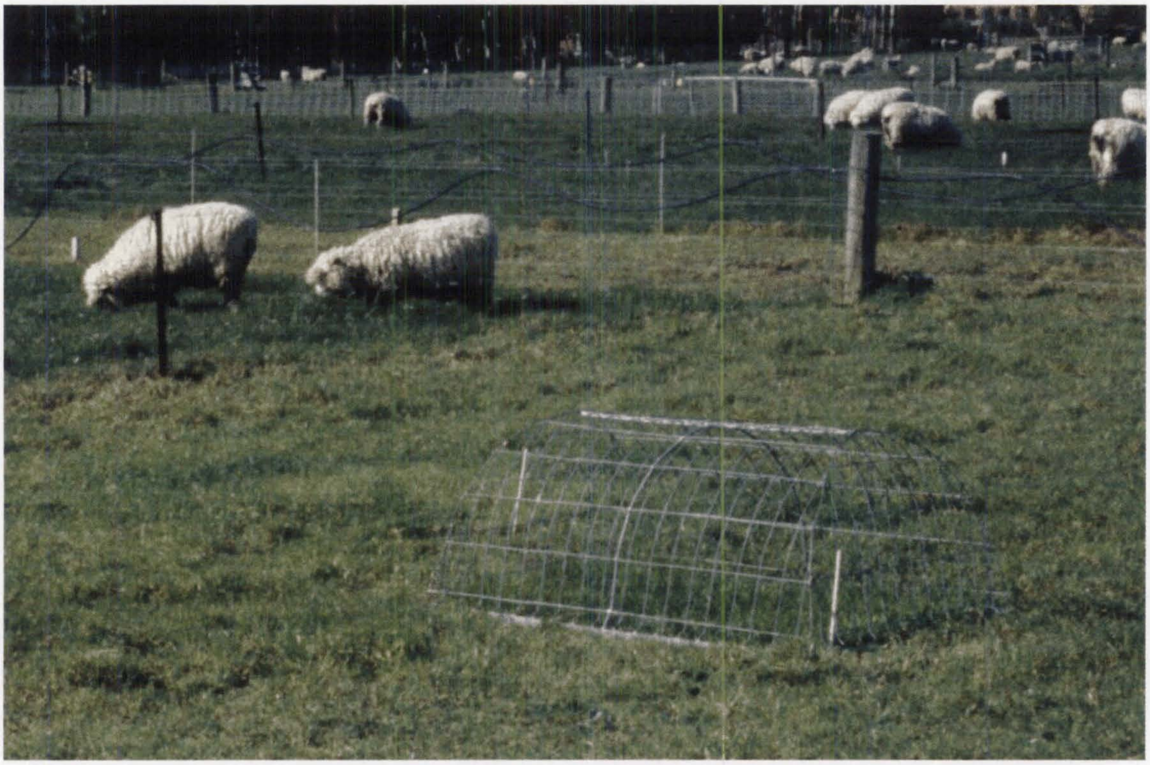


Plate 4 Exclosure cage (1.0 m x 1.5 m) on a previously trimmed area where pasture production was measured in block H17 at Lincoln University, Canterbury, New Zealand (October 2000).

Plate 5 Keith Pollock using the capacitance probe to measure pasture production from a previously trimmed area under an exclosure cage in block H17 at Lincoln University, Canterbury, New Zealand (October 2000).

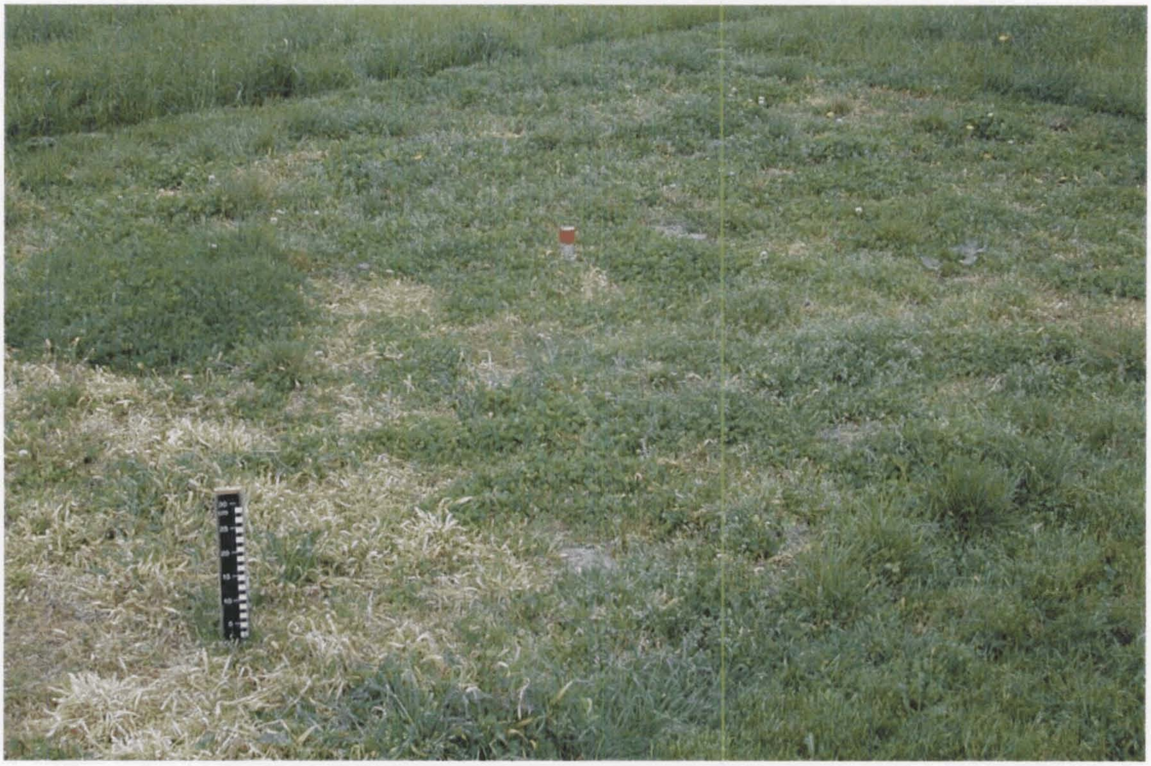


Plate 6 4-year-old sown monoculture of 'Grasslands Demand' white clover under non-irrigated conditions in Iversen 10 at Lincoln University, Canterbury, New Zealand. Photo taken in November 2003. Note the neutron probe access tube in the centre of the 6 m x 4.2 m plot.

Plate 7 4-year-old sown monoculture of 'Endura' Caucasian clover under non-irrigated conditions in Iversen 10 at Lincoln University, Canterbury, New Zealand. Photo taken in November 2003. Note the neutron probe access tube in the centre of the 6 m x 4.2 m plot.

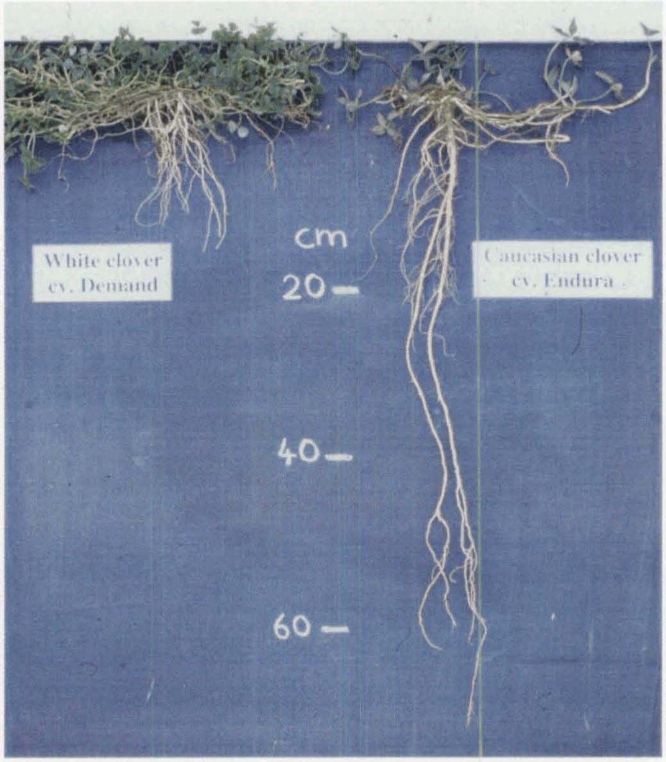


Plate 8 'Grasslands Demand' white clover and 'Endura' Caucasian clover 9 months after sowing in November 1999 in Iversen 10 at Lincoln University, Canterbury, New Zealand.

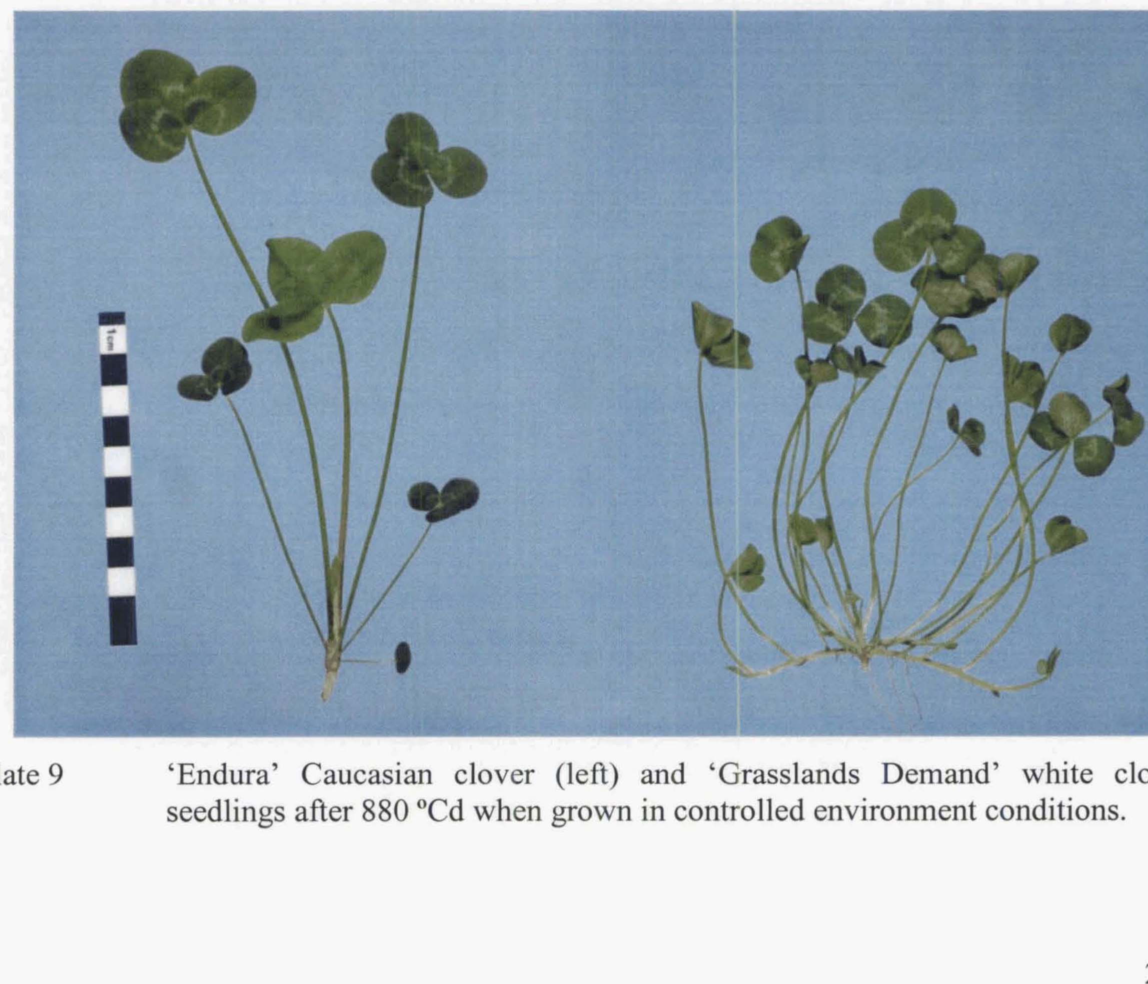


Plate 9 'Endura' Caucasian clover (left) and 'Grasslands Demand' white clover seedlings after 880 °Cd when grown in controlled environment conditions.

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