

The vegetative and reproductive development of balansa clover

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The vegetative and reproductive development of balansa clover (*Trifolium michelianum* Savi.) were quantified in relation to the environmental drivers of each phenophase in field and controlled environments. In a grazed experiment over 6 years, balansa clover sown with cocksfoot (*Dactylis glomerata*) contributed 1.6 t DM/ha/year, or ~20% of the total DM production. However, grazing management for increased seed production during flowering in the establishment year strongly influenced balansa clover regeneration. The earliest closed plot (September) averaged between 2.2 and 4.3 t DM/ha/year of balansa clover across all six years.

In an incubator, balansa clover required 29 °Cd for germination with an optimum temperature of 14 °C and a maximum of 40 °C. The base temperature for germination was 0 °C. A field experiment determined that 38 °Cd were required for emergence with an optimum soil temperature (T_{opt}) of 8.5 °C. The time from emergence until the first leaf appeared, the phyllochron and timing of axillary leaf appearance were compared with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens* L.). The rate of each was found to increase linearly with temperature. The balansa clover cultivar 'Frontier' required 97 °Cd from sowing for the first leaf to appear, had a phyllochron of 47 °Cd and secondary leaves appeared after 490 °Cd. For each vegetative stage, the base temperature was 2.5 °C.

The timing of flower appearance depended on the quantity and direction of change of the photoperiod at emergence. A balansa clover plant, cv. 'Bolta', which emerged on 1 December into an increasing photoperiod of 15.6 hours flowered after 574 °Cd ($T_{base} = 2.5$ °Cd) or 58 days after emergence. In contrast, if the plant emerged on 16 January into a similar but decreasing photoperiod it took 1503 °Cd or 227 days to flower. This length of time became progressively shorter until remaining constant

after the shortest day. In contrast, 'Frontier' took a constant 390 and 690 °Cd in increasing and decreasing photoperiods, respectively. The time which an individual inflorescence took from pollination until seeds were physiologically mature was 250 °Cd for both 'Bolta' and 'Frontier'.

The re-establishment of balansa clover each year relied on a large seed set (>1000 kg/ha) in the establishment year. The continued survival of balansa clover would then depend on a similar seeding event within a 4-5 year period to maintain the seed bank. Management considerations for balansa clover persistence and survival are discussed.

Key words: *Dactylis glomerata*, day degrees, dryland, establishment, leaf area, mixed pastures, temperature, *Trifolium michelianum* Savi syn. *Balansae*.

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1 General Introduction

1.1 Background

To find solutions to the lack of legume persistence in summer dry environments within New Zealand, a collaborative research project, 'MaxClover' (Chapter 3), was established at Lincoln University in 2002. The objectives centred on maximising clover production and persistence within a grazed cocksfoot (*Dactylis glomerata*) pasture and form the basis of this current study. One objective was to evaluate alternative legumes that might provide high quality dry matter and fix atmospheric nitrogen for the cocksfoot component of the pasture. The research in this thesis focuses on the annual balansa clover (*Trifolium michelianum* Savi) as one of the four legumes in the 'MaxClover' experiment.

Cocksfoot is sown in summer dry areas of New Zealand because it competes strongly for available moisture and provides drymatter (DM) for animal grazing when traditional ryegrass (*Lolium perenne*) based pastures are less productive (Mills *et al.*, 2006). However, cocksfoot is frequently nitrogen deficient in a pasture (Peri *et al.*, 2002) which can lead to low grazing preference (Edwards *et al.*, 1993). This can be improved by increasing the available nitrogen in cocksfoot pastures through inorganic nitrogen fertilizers or the successful integration of a legume. The most commonly sown legume in New Zealand is white clover (*Trifolium repens* L.). However, this is unsuited as a companion species for cocksfoot because both compete for limited available water in the dry summer months. Also, the tap root of white clover dies off after 18-24 months (Brock and Caradus, 1996; Knowles *et al.*, 2003) which leaves white clover susceptible to droughts, typical of summer dry areas of New Zealand.

Balansa clover is a top flowering Mediterranean annual legume that is sown in over 1.5 million ha in Australia (Craig and Ballard, 2000). To date there has been no work published on its possible role and performance in New Zealand agricultural systems. The initial results in the first two years of the 'MaxClover' experiment indicated that it had potential as a pasture legume to compliment cocksfoot based pastures.

After sowing in 2002, balansa clover produced 30% of the 10 t DM/ha in 2004/05 and 8 t DM/ha in 2005/06 across four plots with different spring grazing closing dates. In spring 2004, hogget liveweight gain averaged 300 kg/ha from these pastures and was over 450 kg/ha in 2005 (Brown *et al.*, 2006). Based on these two years of positive results from the 'MaxClover' experiment, the present study was initiated to examine the elements that contributed to the success of balansa clover in these cocksfoot pastures. However, in subsequent years the average clover content decreased, so this research also aimed to explain this decline.

The lifecycle of balansa clover is the main area of investigation. Initial research focuses on quantification of vegetative development, namely, emergence and leaf appearance rate in growth rooms. This is followed by analysis of reproductive development and includes the time of flowering and the development of flowers, pods and seeds in a field experiment. After quantifying these stages of physiological development, seedling recruitment from the seed bank was examined across two seasons in the field and comparisons made with other species.

The structure of the thesis is shown in Figure 1.1. Chapter 2 is a review of the literature that introduces balansa clover and the environmental mechanisms that drive vegetative and reproductive development. In Chapter 3, information gathered from a six year dryland grazing experiment at Lincoln University is presented. Chapter 4 presents results from a detailed growth room experiment and a parallel field experiment. The timing and rate of development of reproductive structures are presented in Chapter 5. In Chapter 6, seedling recruitment from the seed bank is reported from a field experiment and the influence and consequences of hardseededness are discussed. Chapter 7 is a general discussion on the implications of this research.

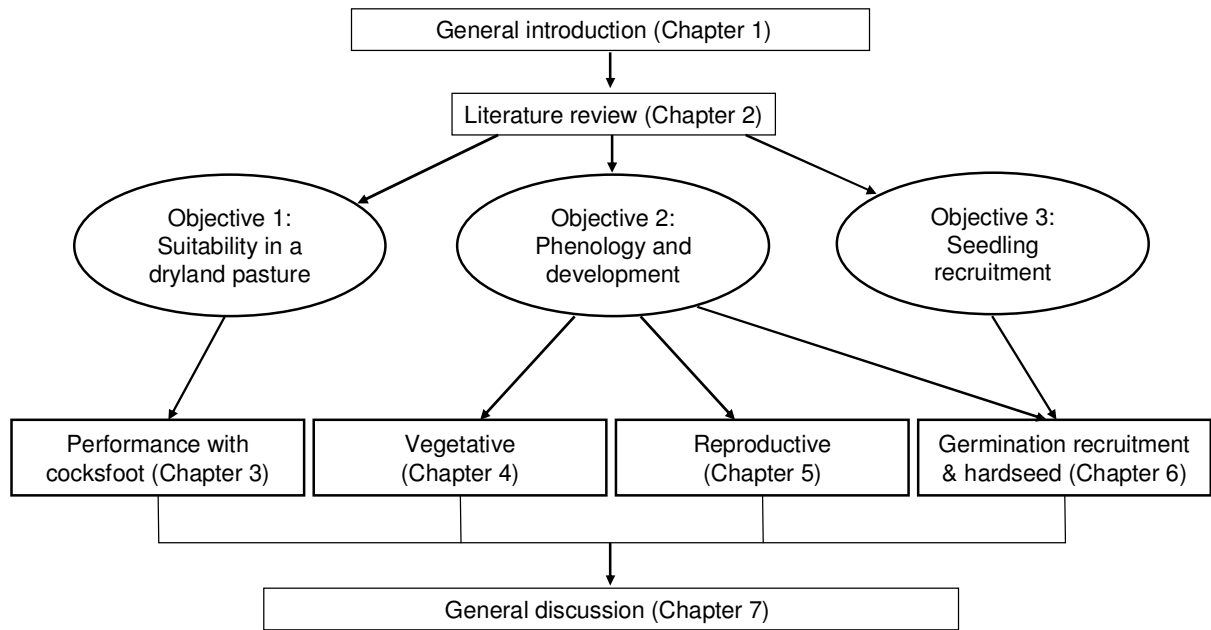


Figure 1.1 Thesis structure

1.2 Research objectives

The aim of this research was to develop best management practices for balansa clover in a grazed pasture system based on an understanding of its key growth and development characteristics. To achieve this aim, several experiments were undertaken with the following objectives:

1. To determine the suitability of balansa clover as a component in a mixed perennial dryland pasture.
2. To define the timing and rate of the phenological stages of balansa clover including germination, emergence, leaf appearance, flowering and seed maturity.
3. To examine the pattern of seedling recruitment of balansa clover over time.

2 Literature review

2.1 Introduction

2.1.1 Background

Legumes are an important component of the dryland (unirrigated) pastoral system in New Zealand. They fix atmospheric nitrogen (N), improve sward quality and therefore increase the performance of grazing animals and compliment grass growth patterns throughout the year. However, New Zealand's most commonly sown legume, white clover (*Trifolium repens* L.), lacks persistence in low rainfall areas (Langer, 1990; Caradus *et al.*, 1995). Knowles *et al.* (2003) reported white clover populations dropped by 75% in Mid-Canterbury when rainfall between November and May dropped to 200 mm from the 373 mm long term mean.

In New Zealand, 2.8 M hectares of land, or about 10.7% of the total land area, receives less than 800 mm of annual rainfall and is consistently prone to summer dry conditions (Brown and Green, 2003). Areas of New Zealand such as north facing hill slopes and land with free draining soils are also prone to periods of limited production due mainly to summer drought. In these areas, rainfall is inadequate to supply all of the water a pasture requires to grow at its maximum potential rate throughout the year. The difference between moisture supply and moisture demand from a crop or pasture is qualified by the potential soil moisture deficit (PSMD). In dryland areas, typical of the east coast of the South Island, PSMD can range from 120 to 500 mm per year (Salinger, 2003). This means up to 500 mm of additional water (irrigation) would be required to maintain field capacity. In New Zealand, PSMD is calculated from 1 July to 30 June the following year and is used to indicate an approaching dry period when soil moisture deficit will limit pasture production. A PSMD above 100 mm has been shown to limit pasture production, and when PSMD is ≥ 150 mm the decrease is severe (McAneney *et al.*, 1982). Furthermore, the maximum PSMD in much of dryland New Zealand is predicted to increase by the year 2050 because of a decrease in annual rainfall (Salinger, 2003).

The traditional growing season in these areas starts as spring temperatures rise and is then usually restricted by a variable summer moisture deficit. Autumn rainfall then re-wets the dry soils which leads to pasture recovery and annual legume re-establishment prior to low temperatures restricting winter growth. The white clover taproot dies after 18-24 months (Brock and Caradus, 1996) so it cannot be relied on in these summer dry conditions. This has led to the search for alternative legumes that are adapted to maintain high quality production in these dry environments.

Pasture species in dryland areas use several strategies to minimise the impact of drought. For example, the persistent tap root of Caucasian clover (*Trifolium ambiguum* L.) enables it to reach moisture deeper within the profile than white clover (Black and Lucas, 2000; Black *et al.*, 2003b). Similarly, lucerne (*Medicago sativa*), chicory (*Chichorium intybus*) and red clover (*Trifolium pratense*) have all been proposed as drought tolerant due to their deep tap roots (Brown *et al.*, 2003). Large perennial root systems maximise moisture extraction from the profile in the dry summers but can limit first season production as the root system is developed (Black, 2004). Of these, lucerne has been shown to be the most productive and persistent (Brown *et al.*, 2006). A limitation of lucerne is the need for it to be rotationally grazed at all times (Moot *et al.*, 2003b). This restricts its use in early spring when ewes and lambs are usually set-stocked in New Zealand. This can create a potential feed gap immediately prior to and during lambing in these dryland systems. This period may be alleviated by the inclusion of winter-active annual clovers or high quality Italian-type ryegrasses. The life cycle of an annual legume means the plant produces seed and dies in the late spring before the onset of drought. It subsequently re-establishes from seed in the seed bank in the following autumn season. Thus, annual plants avoid the summer drought as seeds.

Subterranean clover (*Trifolium subterraneum* L.) has been promoted in New Zealand as the most suitable annual legume for this purpose (Morley, 1961; Smetham, 2003). It has been used extensively throughout Australia where summer drought is more severe and winter temperatures are milder than in New Zealand (Dear, 2003). Subterranean clover gained popularity because it is tolerant of hard grazing, including set-stocking, and there are several cultivars available with different flowering times to suit a range of farm systems (Nichols *et al.*, 1993). Subterranean

clover is a high quality animal feed, with dry mature herbage maintaining a dry matter digestibility of up to 60% and is adapted to permanent pastures in New Zealand (Widdup and Pennell, 2000; Smetham, 2003). Subterranean clover and balansa clover (*Trifolium michelianum* Savi) both have a nitrogen content of $\geq 3.5\%$ between March and October ($\geq 40\text{mm}$ above soil) (Kelly and Mason, 1986).

At the end of its annual life cycle, subterranean clover sets seed into the soil near to the surface. This action makes it somewhat tolerant of grazing throughout flowering but also means that harvesting seed requires specialised equipment. The harvesting process is prone to cause soil losses during wind erosion events. Also, the frequent contamination of subterranean clover seed lines with soil limits importation of the seed into New Zealand (Pers. Comm., Richard Green, Agricom (N.Z.) Ltd., Ashburton, 2005). An alternative annual legume is the top flowering balansa clover (*Trifolium michelianum* Savi). It has fewer seed production and contamination problems. Australian interest in balansa clover is driven, in part, by the fact that it can be harvested without specialised machinery (Dear *et al.*, 2002).

Currently, aerial seeding annual clovers, like balansa, are not well represented in New Zealand due to a lack of industry and research support, the high cost and availability of seed and the limited range of cultivars available (Moot *et al.*, 2003a; Rolston, 2003). Little New Zealand or international information exists on how to utilise these species on-farm or best management practice guidelines for maintaining them in a permanent pasture sward under grazing. Developing these guidelines requires detailed investigation of the growth and development of the species which includes defining requirements for germination, emergence, leaf appearance, flowering and seed set. Answering the basic physiological questions about the development of balansa clover was the main aim of this research. It provides the scientific basis for the basic species and management recommendations that follow which are provided to improve balansa clover's potential in commercial situations.

In this review of literature there is an introduction to cool season annual clovers and a description of the current understanding concerning mechanisms behind their growth and development. Particular attention is given to the quantification of the rate of development, using relevant examples.

2.2 Annual legumes

By definition, annual legumes do not expend energy developing perennial root structures and partition a larger proportion of carbon synthate into above ground shoots than perennial species (Thomas, 2003).

In a dryland experiment on the east coast of New Zealand, subterranean clover produced ~60% of its yearly production in the spring (August – October) period (Brown *et al.*, 2006). In contrast white clover, produces at its highest rate in summer, by which time soil moisture is often depleted, reducing maximum yield (Rickard and Radcliffe, 1976; McAneney *et al.*, 1982).

2.2.1 Balansa clover

Balansa clover (Plate 2.1) is a winter active, out-crossing, indeterminate, top flowering annual legume that is naturally distributed around the Mediterranean region in areas with rainfall ranging from 350 to >600 mm (Craig and Ballard, 2000). The seed germinates with autumn rains and the plant then grows until seed is set in the late spring/early summer. The plant survives the dry summer as a small seed (0.8 – 1.2 mg/seed) with up to 98% hardseededness (Craig, 1998). By comparison, the seed weight of subterranean clover is ~7 mg/seed. Balansa clover is tolerant of saline soils (Rogers and Noble, 1991), poorly drained or water logged soils (Snowball, 1994) and a pH of 5.5 – 8.0 (Bennett and Bullitta, 2003). Balansa clover nodulates successfully with a range of legume inoculants, with favour being given to commercial Group CS (WSM409) (Craig and Ballard, 2000; Ballard *et al.*, 2002).



Plate 2.1 'Frontier' balansa clover harvested on 9 March 2009. Plants, from left to right, emerged on ~18 December, ~18 January, ~12 February and ~22 February.

Balansa clover is considered one of the annual legume species most likely to succeed in the dry east coast environment (Pers. Comm., Keith Widdup, AgResearch Ltd., Lincoln, 2005). It offers adaptive characteristics that may compliment the other species sown in the summer-dry regions of New Zealand. The possible role of balansa clover, like all new species, is that it would compliment subterranean clover by colonising different soil type, drainage or pH niches within a sward (Dear *et al.*, 2002; Zhang *et al.*, 2004). It would then offer high quality feed of adequate quantity particularly during periods of high demand, such as autumn and spring.

There are six commercially released cultivars of balansa clover with a range in maturity date. 'Frontier' and 'Enduro' are the earliest flowering cultivars and are suited to low rainfall areas (<350 mm/year). 'Frontier' is a selection of 'Paradana' germplasm (Craig *et al.*, 2000). 'Paradana' and 'Taipan' are considered to be suited to areas of 500-600 mm/year rainfall. 'Bolta' and 'Viper' are the latest flowering cultivars and suit environments receiving more than 600 mm/year rainfall. However, these suggestions are based on empirical observations and calendar days from Australian research (Craig and Beale, 1985; Craig and Ballard, 2000) and may not

necessarily transfer to New Zealand climates and locations. In southern Australia, the cereal cropping rotation has relied on temperate annual legumes and medics as the main source of nitrogen input for over 60 years (Dear, 2003) with balansa clover used for grazing, hay, silage or green manure in over 1.5 M ha (Craig and Ballard, 2000). Balansa clover is used as a monoculture in these systems so hardseededness ensures it re-establishes the year after the cropping phase. The balansa clover can then be hard grazed after autumn emergence through to the beginning of flowering in the spring when it is spelled to reseed. A hard 'clean-up' grazing in early summer assists hardseed breakdown (Quinlivan, 1966) and establishment of the following cereal crop (Craig and Ballard, 2000).

'Paradana' was the first commercially released cultivar (Craig and Beale, 1985) and started to appear in the literature when P. I. Jansen began publishing work from his PhD thesis under the supervision of R. L. Ison. Their published research focused on seed quality (Jansen, 1995b, 1995a) and recruitment (Jansen and Ison, 1994a, 1994b, 1995, 1996; Jansen *et al.*, 1996). The implications of their work form the basis of most of the current recommendations for balansa clover.

'Paradana' balansa clover seed has an optimum temperature (T_{opt}) for germination rate of 15 °C (Jansen and Ison, 1994a). Final germination rate and percent became compromised when temperatures were above 20-25 °C and seeds did not germinate above 35 °C. The seed was found to exhibit high-temperature dormancy that may convey protection from an 'out of season' summer rainfall events (Knight, 1965; Evers, 1980) (false strike, Section 2.10.2.2). High-temperature embryo dormancy prevents germination when moisture supply is adequate and reduces final germination percent as temperatures rise above a critical level.

The largest seeds of 'Paradana' were from the earliest formed inflorescences and were more numerous and had higher hardseed content than those formed in later inflorescences (Jansen, 1995a). The implication is that the balansa clover plant puts more resources toward ensuring the seeds of the first inflorescence on each plant had the highest chance of being viable and useful for survival. Seeds of the first inflorescence may also have a higher level of hardseededness (Halloran and Collins, 1974). The seeds of 'Paradana' ranged in colour from black, red brown and light

brown but the relationship between seed colour and hardseededness was found to be inconclusive and was not suggested as a way to predict the hardseed content and future survival (Jansen, 1995b). The amount of hardseed present at the beginning of the autumn break was only a minor factor in determining persistence in balansa clover compared with seedling establishment (Jansen *et al.*, 1996). Smetham (2003) reported that subterranean clover required 1000 seedlings/m² in autumn for 'agricultural success'. The critical number of seedlings for balansa clover will be different for different growing environments and is dependent on plant development, specifically seedling establishment (Black and Lucas, 2000; Hurst *et al.*, 2000).

Jansen *et al.* (1996) then published a model that simulated the persistence of a pure sown balansa clover stand allowed to regenerate over three years. The model collated other work with seed losses over time from a known seed bank in central west New South Wales (NSW) (Jansen and Ison, 1995). They accounted for losses due to germination, seedling death, and predation by ants, grazing animals and fungi. Out of season rainfall leading to seedling death (false strike) was the most important factor leading to seed loss from the seed bank and accounted for 76% of all seed lost over summer (13% of total seed).

Jansen *et al.* (1996) also found that a pure stand of 'Paradana' balansa clover sown in NSW persisted from year to year through self-regeneration. The largest single factor influencing persistence of a balansa clover stand was the level of annual seed production. In that environment (524 mm/year rainfall evenly distributed but variable), seed production ≥ 400 kg/ha/year for 'Paradana' was able to overcome failed seed production one in every two years. They also suggest a longer growing season in a more reliable climate would mean seed production was required only every third year. This emphasizes the importance of selecting the correct cultivar for the environment and the importance of allowing balansa clover to flower. Low seed production for any reason would compromise persistence.

Dear and Coombes (1992) and Dear *et al.* (2002) found that balansa clover was weakly competitive as a seedling compared with subterranean clover. Balansa clover was unable to restrict subterranean clover growth in mixed swards even when the balansa produced more than 3000 seedlings/m² with positive flowering and hardseed

levels (Dear *et al.*, 2002). The authors concluded that grazing at establishment may have been able to control competition by preventing the small seedlings from being shaded out. Quantification of the characteristics that define seedling development may enable more critical and analytical assessment of the competitiveness of balansa clover to be made. It was an objective of this thesis to quantify seedling development stages as a component of seedling competitiveness.

2.3 Plant development

Plant development defines the stages of phenological change in a plant. The development stages that influence seedling competitiveness include: germination, emergence and appearance of the first main-stem trifoliate leaf, as well as the rate of appearance of successive primary leaves (phyllochron) and the appearance of axillary vegetative structures (secondary development) (Hurst *et al.*, 2000; Black *et al.*, 2006b).

2.3.1 Thermal time

For many species, the rate of germination (Arnold and Monteith, 1974), appearance of the first trifoliate leaf, phyllochron (Moot *et al.*, 2003a) and the duration from pollination to maturity (Hyde, 1950) are linearly related to temperature. In contrast, the switch from vegetative to reproductive development may be primarily quantified by temperature but is often modified by photoperiod and vernalisation requirements, as with subterranean clover (Aitken, 1955; Major, 1980). Defining these relationships provides transferable coefficients that can be used outside the site of origin. Quantifying the thermal time (synonymous with degree days) requirements for each of these phenological development stages for balansa clover was a main objective of this research (Chapters 4, 5 and 6).

2.4 Germination

Once seeds imbibe water, the process of germination through enzyme mobilisation of seed reserves begins. The rate of germination shows a strong relationship with mean temperature for both annual and perennial pasture species (Arnold and Monteith,

1974; Charlton *et al.*, 1986; Hampton *et al.*, 1987; Norman *et al.*, 1998). A linear relationship indicates that the base temperature and thermal time (Tt) requirements for each development phase can be calculated from the least squares regression coefficients (Angus *et al.*, 1981) where:

Equation 2.1 $1/t(\text{rate}) = a + bT$ $T_{\text{base}} < T < T_{\text{opt}}$

Where t is time to germination (°Cd), a is the y-axis intercept and b is the slope coefficient. T is temperature, where T is between base temperature (T_{base}) and optimum temperature (T_{opt}).

T_{base} is the minimum temperature at which thermal time is accrued towards each phenophase. With regards to leaf appearance for example, below the T_{base} plants will not develop additional leaves. T_{opt} is the optimum temperature, at which thermal time is accrued at the fastest rate for each phenophase. For example, at T_{opt} , leaves appear at the fastest rate. T_{max} is the maximum temperature at which thermal time is accrued towards each phenophase. Above the T_{max} plants will not develop additional leaves.

In Equation 2.1, the rate of seed germination ($1/t$, days⁻¹) is defined as the inverse of the time required to reach a certain percentage of germination, in most cases 75% (Moot *et al.*, 2000). The rate of germination increases linearly with temperature from an extrapolated base temperature (T_{base} , $-a/b$), at which the rate is zero, up to a maximum rate at an optimum temperature (T_{opt}). Temperatures above the optimum affect germination rate adversely. The rate of germination decreases linearly until the rate of germination reaches zero again at the maximum temperature (T_{max}) (Moot *et al.*, 2000). The thermal time interval for each phenophase (phase of phenological development) can then be calculated as:

Equation 2.2 $Tt = 1/b$ $T < T_{\text{opt}}$

For many legumes of Mediterranean origin, base temperatures range from 0 to 5 °C, optimum temperatures from 16 to 22 °C and maximums from 30 to 40 °C (Angus *et al.*, 1981; Norman *et al.*, 1998). The ecological significance of these ranges is

important. For balansa and Persian (*Trifolium resupinatum* L.) clovers, temperatures above 30 °C following summer or autumn rain will prevent germination (Jansen and Ison, 1994a). This short-term high temperature dormancy reduces the risk of 'false strike' by preventing rainfall in summer inducing germination.

Both the base and optimum temperatures for germination for a range of pasture species have been calculated, including white clover, perennial ryegrass and Caucasian clover (Black *et al.*, 2003a). In absolute terms, germination of these perennial species in incubators took longer at low temperatures of 0 to 5 °C (Charlton *et al.*, 1986). An increase in temperature led to a decrease in the number of days taken for germination. This then formed a linear relationship between temperature and the rate of germination. These individual relationships can then be used to define the cardinal points of T_{base} , T_{opt} and T_{max} and thermal time for germination.

Plant development is a biological process and the threshold temperatures of T_{base} and T_{max} are only statistical estimates of the true processes involved (Angus *et al.*, 1981). The plant experiences temperature changes throughout the day at the shoot apical meristem (Peacock, 1975), but thermal time calculations assume a single value for temperature each day. Mean temperature in the field is calculated by interpolating daily sinusoidal temperature variation. The true plant development rate does not react linearly when mean calculated temperature approaches T_{base} or T_{max} (Bonhomme, 2000). For example, when the mean air temperature of day x is the same as the calculated T_{base} , approximately half of day x is spent above the mean temperature. During half of the day, the plant accrues thermal time towards development that is not accounted for in Tt calculations. Because of this, a two-part linear regression or a curvilinear regression as temperatures approach the cardinal points may be more appropriate to describe the true response of plant development to mean daily temperature (Wilson *et al.*, 1995).

Another way to account for this phenomenon is to use a single value for daily temperature that more accurately accounts for what the plant experiences. That is, change the daily thermal time accumulated to better represent the daily sinusoidal fluctuations (Jones and Kiniry, 1986). Jones and Kiniry (1986) used an equation to translate maximum and minimum daily temperatures into eight three-hour mean

temperatures that are then integrated to give a single value (Equation 4.2, p50). This method was used in the present study and gave reliable results.

To enable direct comparison of the Tt requirements for germination within and between species, additional regression analysis of rate against temperature can be performed with T_{base} set to 0 °C (y intercept = 0) (Moot *et al.*, 2000). Applying this normalisation allows comparisons across species with different base temperatures when grown in sub- T_{opt} field conditions. For example, when T_{base} was set to 0 °C, the Tt requirements for germination of white clover were 40 °Cd; Caucasian clover, 46 °Cd and perennial ryegrass 76 °Cd (Black *et al.*, 2006a). These numbers can then be compared using readily available meteorological data, as reported in many local newspapers.

2.5 Emergence

After germination, seedlings emerge from the soil at a linear rate with thermal time (Moot *et al.*, 2000). Using experimental and published data, Moot *et al.* (2000) estimated the thermal time requirement from sowing to 50% emergence for a range of dicotyledonous and monocotyledonous herbage species, including white clover which was reported to be $T_{\text{base}} = 0$ °C and $Tt = 150$ °Cd; and perennial ryegrass, $T_{\text{base}} = 0$ °C, $Tt = 160$ °Cd.

2.6 Primary leaf production

After emergence of the cotyledons and 'spade' leaf, trifoliolate leaves appear sequentially on the main stem. The period from emergence until the appearance of the first mainstem leaf is not constant in calendar days, but becomes uniform when using thermal time. Similarly, the phyllochron is usually a constant interval when thermal time is accounted for (Gallagher, 1979). The majority of the published work on phyllochron has been based on field crops e.g. Gallagher (1979) with wheat or Kirby *et al.* (1982) with barley.

2.6.1 Phyllochron

The phyllochron defines the chronological time between the appearance of each successive mainstem leaf. Phyllochron is often presented in thermal time as a measure of plant development independent of calendar days or environmental variables. For example, the timing of anthesis in wheat is often described in terms of the number of mainstem leaves present (Jamieson *et al.*, 1995a), which is useful in the field when measures of temperature and thermal time may be impractical. Phyllochron has been shown to be consistent for at least the first five mainstem leaves for several herbage species (Black *et al.*, 2006a; Lonati *et al.*, 2009). Jamieson *et al.* (1995b) showed that temperature alone was the driver of phyllochron and that any 'change' in phyllochron from plants sown at different times was due to a failure to identify the temperature the apex of the plant perceived. They showed that the underlying estimates of phyllochron were wrong when based solely on air temperature as published by authors such as Baker *et al.* (1980) or Masle *et al.* (1989). Jamieson *et al.* (1995b) suggested that the other models which indicated that phyllochron changed by sowing date were only accounting for errors in the temperature measurement.

2.7 Secondary leaf production

Secondary leaf appearance is synonymous with the timing of the first axillary leaf and defines the time that an establishing plant begins to increase the number of leaves at an exponential rate. During the establishment phase, plants produce a canopy and capture incoming solar radiation with a rate which is linearly related to leaf number (Brown *et al.*, 2005). Therefore, plants that produce secondary leaves earlier than other species may have a competitive advantage for capturing light.

For example, Caucasian clover is weakly competitive with white clover at establishment because the thermal time requirement from sowing to secondary leaf appearance is higher at ~990 °Cd compared with ~430 °Cd for white clover. This is despite similar thermal time requirements for germination, emergence and the phyllochron of primary leaves. The often seen failure of Caucasian clover to establish within a pasture is because it is unable to develop adequate leaf architecture to capture sufficient solar radiation before seedling reserves run out (Black *et al.*,

2006b). Because these seedling characteristics were quantified, Black *et al.* (2006a) and Hurst *et al.* (2000) were able to identify the areas of weakness that Caucasian clover had at establishment and designed management recommendations to overcome them.

2.8 Reproductive development

Unlike plant growth, which is the accumulation of dry matter as the product of photosynthesis, plant development encompasses a complex series of events that are difficult to describe with regards to a single, unified measurement (Landes and Porter, 1989). Because of this, there is a need to divide the development of plants into identifiable stages that can each be described in terms of their response to environmental variables.

2.8.1 Development scale

Because management for seed production is crucial for continued balansa clover presence in the sward, this study divided reproductive growth into several easily defined phases. Previous work on the visual development of reproductive structures for other legumes was taken into account in the development of the new scale to describe inflorescence development (Section 5.2.1.2).

Work by Kalu and Fick (1981) summarised the literature describing lucerne reproductive development. They showed that existing stage classifications were based on the flowering status of the crop from a macro level, e.g. Nelson (1925). Kalu and Fick (1981) clarified the later stages of individual inflorescence development through the change in seed colour as they mature. No existing classification was able to satisfactorily describe the development of the balansa clover inflorescence. Specific inflorescence morphology, including prefloral stages and organ formation and differentiation, was beyond the scope of this research, and was assumed to progress similarly to those described by Tucker (1987), Carlson (1966) and Thomas (2003) for legumes species in general and the more recent work with *Pisum sativum* by Ferrandiz *et al.* (1999).

2.8.2 Floral initiation

The timing of flowering is driven by environmental and endogenous plant processes, including temperature, photoperiod and circadian rhythm. When wheat (*Triticum aestivum*) was moved across latitudinal boundaries from Central USA to Scandinavia it led to variations in flowering patterns (Schubeler, 1880). Thomas and Vince-Prue (1997) cite two independent efforts as the first mention of the influence of photoperiod (Pp) on the induction of flowering in the literature as Tournois (1912) published in France and Klebs (1910) published in the UK.

In the 1930's, Chailakhyan, a Russian scientist, performed a number of experiments that isolated plant parts and showed that it was the leaf that senses the environmental stimulus for flower induction (Aksenova, 2002). Chailakhyan was the first to suggest that the signal is then transported to the shoot apical meristem for interpretation. He postulated the transportation mechanism to be a hormone that he named 'florigen'. Recent work has proposed that the gene *FLOWERING LOCUS T* (*FT*) or its product is the transport mechanism and travels through the phloem to the apical meristem (Zeevaart, 2008).

The shoot apical meristem must change from a juvenile, non-receptive state, to a mature state, able to interpret incoming signals and initiate flowering (Lang, 1965; Poethig, 1990). Because of this, some plants are said to have a juvenile phase where flowering cannot be initiated (Adams *et al.*, 2001), such as in lucerne (Robertson *et al.*, 2002).

2.8.2.1 Photoperiod

Garner and Allard (1920) showed that flower initiation was dependent on the length of day (photoperiod (Pp)), and that plants do not all react the same way to daylength. Following the separation of daylength from other environmental variables the importance of photo period in initiating flowering was highlighted.

Many plants demonstrate a change in the time of flowering if they are grown in environments with a different photoperiod. Balansa clover (Jansen, 1995a) and subterranean clover (Aitken, 1955) are both described as long-day plants. This

means that as photoperiod decreases below a critical photoperiod, it takes a longer time for flowers to initiate. The time of flowering and rate of change are determined genetically and have been shown to be cultivar specific for many field crops (Major, 1980) and for lucerne (Major *et al.*, 1991).

2.8.2.1.1 Perception of change in direction of daylength

Working with wheat development models, Brooking *et al.* (1995) showed that plants are able to perceive the direction of change in photoperiod. By implication, this means that in addition to being able to a) discriminate between light and dark and b) measure the length of these periods (Thomas and Vince-Prue, 1997), plants also have the ability to c) perceive changes in the direction of daylength. Plants detect the direction of change in photoperiod through the use of a circadian light cycle (Millar, 2004). This endogenous mechanism, known as circadian rhythm, is able to influence genes and proteins to change their expression (Tremblay and Colasanti, 2001). One of these proteins is called CONSTANS and was discovered to promote flowering in *Arabidopsis* (Putterill *et al.*, 1995). Production of the CONSTANS protein is mediated by the output from the mechanism that records changes in circadian rhythm within a plant. It may be this action that governs the ability of the stem apical meristem to commence flowering (Section 2.8.2).

The concept of direction of photoperiod change influencing flowering is not without precedence. Clements (1968) presented work with sugar cane (*Saccharum spp.*) that showed an increasing photoperiod would induce flowering where similar absolute but decreasing photoperiod would not. The most common critique of this type of work, however, is that successive stages in floral development may have individual photoperiod length requirements (Thomas and Vince-Prue, 1997). If later stages of flower initiation are obligative long day processes, which have a minimum critical photoperiod for flowering, decreasing photoperiod below the critical limit would halt flowering. Brown *et al.* (2005) also showed a change in phyllochron within lucerne crop regrowth that changed with photoperiod. They suggested that the phyllochron remained constant, but the expression of it was limited because the majority of the assimilate was being partitioned away from above ground structures to replenish storage organs.

2.8.2.2 Endogenous cues

In some plants floral initiation is solely dependent on endogenous, internal cues. Other plants use a combination of endogenous and environmental stimuli to initiate flowering. Endogenous cues may include plant size, leaf number or hormone balance (Bernier *et al.*, 1981) and the maturation of the apical meristem itself (Section 2.8.2).

2.8.3 Inflorescence development

The maturation phase of an inflorescence is the period between flowering or pollination and seed maturity and is linearly related to temperature (Quinlivan *et al.*, 1987; Poethig, 1990). Work by Jansen (1995a) into the duration of flowering in balansa clover showed 'Paradana' took only 11 days from flower initiation until physiological maturity at 30/25 °C, or ~300 °Cd, and 7 weeks at 18/13 °C, or ~700 °Cd. He gave no temperature thresholds, but instead proposed an empirical formula for his environment (Equation 2.3)

Equation 2.3 Maturation phase = $83.5 - 2.41 \times \text{temperature} (^{\circ}\text{C})$

2.9 Seed production

Survival and persistence within and between seasons for annual species is promoted by producing sufficient seed, a suitable pattern of seed coat impermeability (hardseededness) and seed dormancy breakdown, the survival of seedlings through to seed production, seed dispersal and germination (Cocks, 1996).

Balansa clover is a prolific seeding species, producing 340 million to 1.7 billion seed/ha (Jansen and Ison, 1996; Craig, 1998). The seed weight is shown in Table 2.1. Modelling work by Jansen *et al.* (1996) based on field experiments showed 'Paradana' balansa clover persisted in a permanent pasture of the central west New South Wales, Australia, because of the high number of seeds produced. In the same modelling work, Persian clover (0.65 – 1.4 mg/seed) was unable to maintain a presence in a permanent pasture because it produced less seed.

Table 2.1 Seed weight and annual seed production of 'Bolta', 'Paradana' and 'Frontier' balansa clover and subterranean clover from a range of sources.

	Seed weight (mg)	Seeds/m ²	Seed production (t/ha)	Location	Reference
'Bolta'	1.17	85500	1.0	Sth. Aus.	Craig (1998)
		111000	1.3	NSW	Jansen <i>et al.</i> (1996)
		170000	2.0	Lincoln, NZ	Moot, unpublished
'Paradana'	0.98			Sth. Aus.	Craig (1998)
	0.72			NSW	Jansen (1995a)
	0.72	69000 180000	0.5 (dryland) 1.3 (irrigated)	NSW	Jansen and Ison (1996)
'Frontier'	1.2	42000	0.5	Sth. E. Aus. (Acidic soils)	Dear <i>et al.</i> (2002)
		100000	1.2	Sth. Aus.	Craig <i>et al.</i> (2000)
Sub. clover 'B. Marsh'	3.5-9	15000	1.0	Sth. Aus	Black (1956)

2.10 Seed maturation

Efficient management of an annual clover based pasture post-flowering requires an understanding of seed maturation. Development of viability within a seed can be used to determine the period of time before stock should be reintroduced into the pasture for grazing.

2.10.1 Physiological maturity

Physiological maturity in seeds is defined as the point of maximum dry weight and is the point of maximum seed germination and vigour (Hyde, 1950; Hyde *et al.*, 1959).

The time from pollination/anthesis to maximum seed weight has been defined for several species including wheat (Brocklehurst, 1977), perennial and Italian type (*Lolium multiflorum*) ryegrasses, red clover and white clover (Hyde, 1950; Hyde *et al.*, 1959) and Lotus (*Lotus pedunculatus*) (Hare and Lucas, 1984), but not for balansa

clover. Hyde *et al.* (1959) presented a detailed study of the development of viability and hardseededness and the change in seed weight over time in two perennial clover species. Figure 2.1 shows the increase in dry weight, fresh weight and subsequent moisture content of white clover after pollination. These phases correlate to the change in viability and hardseed percentage in white clover. The whole process, from pollination until maximum seed dry weight took ~25 days. With an assumed base temperature of ~0 °C and a mean daily temperature of ~18 °C (calculated from long term means for the period from the experimental site) during the period from pollination to maturity, thermal time can be calculated from this data. Based on this, the duration from pollination to physiological maturity was ~450 °Cd.

Figure 2.1 also shows the onset of hardseededness (Section 2.10.2) paralleled viability approximately 5 days later, leading to a decrease in germination. This means that while viability is linked to dry weight, maximum germination percent is not.

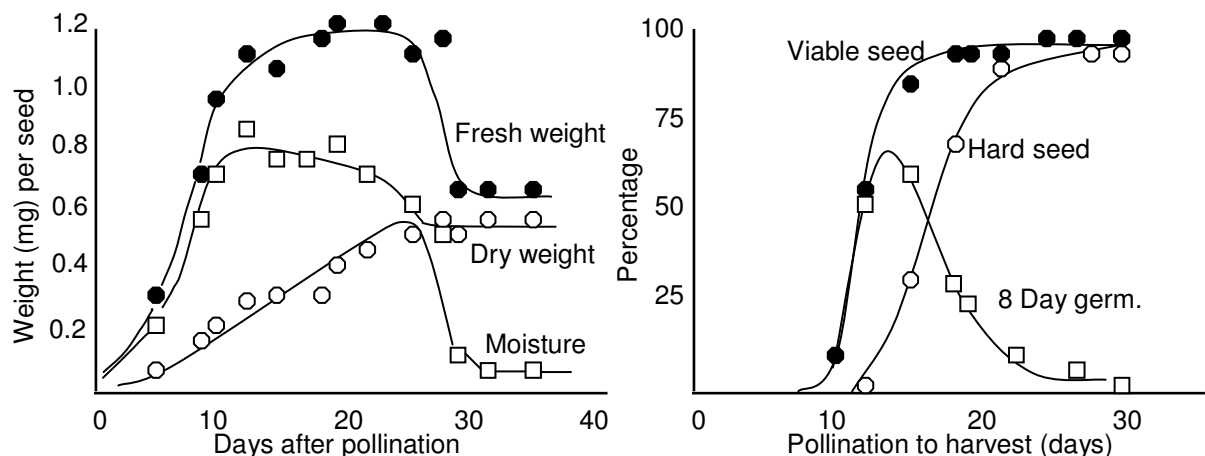


Figure 2.1 (a) Weight changes in white clover seed during development and (b) the effect of stage of development at harvesting on seed viability and hardseededness in white clover seed. From Hyde *et al.* (1959).

2.10.2 Hard seed and dormant seed

Legume seeds have a hard seed coat (testa) that prevents germination by stopping water and gas movement to and from the endosperm. This physical barrier results in hardseededness and is separate from any dormancy seeds may exhibit. The physical impermeability is caused by a suberin deposition that thickens the seed coat surrounding the Malpighian layer (Quinlivan, 1965). The extent of the thickening is

environmentally controlled within each genotype (Quinlivan and Nicol, 1971) and is promoted by moist, cool conditions during seed set (Taylor, 1996; Norman *et al.*, 2002). Increased available moisture during seed growth leads to an increase in thickening and seed coat impermeability in subterranean clover (Gladstones, 1958; Quinlivan, 1971). This physical restraint on germination can affect a temporal dispersal of seed that benefits the plant by preventing all the seed from germinating in the same season or by spreading the period of germination within a season (Smith *et al.*, 1996; Norman *et al.*, 2002). Dormancy is an endogenous barrier to germination. It prevents viable, physiologically mature seeds from germinating in an environment that is otherwise conducive to germination by preventing moisture entering the seed (Quinlivan and Nicol, 1971).

Hardseededness is seen as an important part of Mediterranean annual clover persistence in Australian climates and in ley-crop farming (Jansen *et al.*, 1996; Taylor, 2005). The focus on percent hardseed of subterranean clover at seed set is different between New Zealand and Australia. In Australia, high maximum temperatures and large diurnal fluctuations lead to a rapid breakdown of hardseededness (Quinlivan, 1961, 1966). Therefore it is desirable to have a species that has high initial levels of impermeability. Taylor *et al.* (1991) demonstrated that if annual clover populations in Australian pastoral systems were to be maximised long term, ~40% of seed should soften over the first summer, leaving ~60% hard in the autumn. Conversely, in east coast regions of New Zealand subterranean clover which has a relatively low level of hardseed is recommended (Rossiter, 1978; Smetham and Wu Ying, 1991). Because of the consistent winter rainfall in New Zealand, complete failure of a species due to drought is rare compared with true Mediterranean climates. However, the recommendation for low levels of hardseededness was made for subterranean clover and not a top flowering species like balansa clover. For balansa clover, flowering each year would require removing animals from the paddock during spring, a period of high demand for feed. Because of this, a relatively high level of hardseededness may promote balansa clover survival over multiple years and allow it to be more successfully integrated into a typical New Zealand dryland sheep and beef production unit (Section 2.10.3).

In areas where late summer or autumn rainfall is unreliable, the frequency of a false break is increased (Section 2.10.2.2) and the desirable level of hardseed and dormant seed increases. The climate in the wheat belt of NSW or in Western Australia can also lead to the desiccation of summer-germinated seedlings. Smetham and Wu Ying (1991) point out that where summer rainfall allows such seedlings to survive, as with some areas on the east coast of New Zealand, little hardseed is required for persistence between seasons. However, in a grazed situation, it would be advantageous for top flowering annuals such as balansa clover to survive and persist from season to season from seed stores, rather than require spelling for seed set each year.

New Zealand pastoral farmers have inherited Australia's breeding focus with their desire for high levels of hardseededness. Currently there are no balansa clover cultivars on the New Zealand market that have been bred or selected for New Zealand conditions. The cultivars available through local merchants are 'Frontier' and 'Bolta' balansa clover. These cultivars were developed in Australia and are both described as having 95% hardseed at maturity in early summer, falling to 50% at the break of the season in autumn (Craig and Ballard, 2000). Quantifying the influence of hardseed on long term persistence in a mixed sward was one of the objectives the present study (Chapter 6).

2.10.2.1 Breakdown of hard seed

For hard seeds to germinate the seed coat must be physically broken down to enable water and gas to reach the endosperm. Within each species, the breakdown of hard seed (seed softening) is commonly accepted to be influenced by the environment. The mechanisms responsible for the breakdown of hardseededness in balansa clover are unknown (Jansen *et al.*, 1996) but it is not believed to be based solely on the diurnal temperature fluctuation that drives seed softening in other legumes (Pers. Comm., Clinton Revell, Department of Agriculture and Food, Western Australia, 2008).

In field conditions, the daily alternating temperature cycle causes softening of the impermeable seed coat in a range of legumes (Quinlivan, 1971). The rate of

hardseed breakdown in subterranean clover was originally thought to be dependent on the maximum temperature that the seeds were exposed to (Quinlivan, 1961). It was later shown in incubators to be related to the amplitude of fluctuation (Quinlivan, 1966). The rate of seed softening was the same when the diurnal temperature fluctuation was greater than 15 °C. The gradual increase and decrease of temperature (Taylor, 1981) are believed to expand and contract the testa, leading to the creation of fissures that water can penetrate (Quinlivan, 1961; Ballard, 1976; Hilhorst, 2007).

2.10.2.1.1 Two-stage pre-conditioning

Subterranean clover (Taylor, 1981) and both burr medic (*Medicago polymorpha*) and barrel medic (*M. truncatula*) exhibited an increased hardseed breakdown rate after a period of pre-conditioning (Taylor, 1996). Seeds only became soft during the fluctuating temperature treatments after a period of constant temperatures above 20 °C. The absolute temperature of the pre-conditioning phase, like the diurnal fluctuation itself, was suggested to be genetically controlled and possibly different for different cultivars and species. Temperatures below 20 °C were unable to pre-treat the seeds in the same way. This is therefore another mechanism of adaption towards ensuring seeds germinate at the time of the year when the seedlings are most likely to experience prolonged, favourable growing conditions.

2.10.2.2 False break

Initial late summer or autumn rainfall is not always followed by consistent rainfall for seedlings to survive. This leads to a 'false break' whereby seeds imbibe, germinate and emerge with the moisture from the first rain but die off before further rainfall. Any loss of viable seed from the seed bank through a false break reduces the number of seedlings established at the start of the 'true' season. The number of balansa clover seedlings that survive to produce seeds can be dramatically diminished by a false break (Jansen and Ison, 1995).

2.10.3 Grazing management

Grazing balansa clover during the flowering period compromises seed production. It has been shown that even lax grazing of balansa clover during flowering can reduce seed production by more than 50%, in contrast to only 8% in the prostrate, seed burying subterranean clover (Bolland, 1987). In years/crops when maximum seed production is the priority, stock should be taken out of the paddock before flowering begins and only reintroduced to graze the standing herbage after the seed becomes viable (Bolland, 1987). The date when stock are removed from the paddock and defoliation stops is the closing date. A difficulty for New Zealand farmers is that the time of closing and frequency required for regeneration of seed are unknown. In the only grazing experiment in New Zealand reported to date, balansa clover has shown some promise as an annual species in a grazed system (Brown *et al.*, 2006). Defining optimum closing date was one of the objectives of this present study.

2.11 Seedling competition

When sown in a non-limiting environment, a clover seed germinates, emerges and produces primary and secondary leaves at its maximum rate. However, competition for light, moisture and nutrients and disturbance by grazing, pests, and adverse environmental conditions alter the growth of the plant and decrease productivity (Grime, 1977). When considering several annual clovers, Norman *et al.* (2005) contrasted species which have a large number of relatively small seeds, such as balansa clover (~1.2 mg), with species which produce fewer relatively large seeds, such as subterranean clover (~6.7 mg). A small seeded species, therefore, invests relatively few resources per seed, relying on few seedlings to survive from many produced. A large seeded species, in contrast, invests greater resources per seed, relying on many seedlings to survive from few produced (Norman *et al.*, 2005; Turnbull *et al.*, 2005). Larger seeded species also produce larger seedlings which have greater cotyledon area and produces larger seedlings (Black, 1956, 1958). This may not, however, translate into higher relative growth rates. Some annual clovers, including Balansa clover, have shown that smaller seeds result in a higher relative growth rate compared with larger seeds from the same species (Taylor, 1972a; Dear *et al.*, 2006).

2.12 Conclusions

Balansa clover is a top flowering annual legume from the Mediterranean that may offer an alternative to subterranean clover in summer-dry areas of New Zealand. For balansa clover to become a successful component of a New Zealand pasture, we must understand the relationship between plant development and the environment. This review has presented the methods that have been used to quantify the rate of growth and development in crops and other pasture legumes and how they might be applied to balansa clover.

- The benefits of a new pasture legume like balansa clover are only manifest when it is successfully integrated into a pasture system. The success or failure of an annual legume is governed by its ability to capture incoming solar radiation and produce an adequate amount of seed to persist from year to year. The characteristics that determine the timing of these events are quantified by the rate of plant development.
- Plant development responds to the environment. Germination, emergence, mainstem leaf appearance rate, timing of secondary leaf appearance, timing and duration of flowering, the formation of viable seeds and the breakdown of hardseed are all governed by identifiable and measureable environmental conditions (temperature and photoperiod).
- Successfully quantifying the rate of development relative to the environmental stimulus will improve understanding of the species. This knowledge is imperative for the design of sound management practices.

The aim of this study was to develop basic management strategies for balansa clover within a grazed pasture system, based on an understanding of its key growth and development characteristics. The first of several experiments was a long term, grazed, mixed pasture experiment that was analysed to describe the performance of balansa clover sown with cocksfoot under different management practices. The remainder of the study analysed the life cycle of balansa clover, chiefly to quantify those development characteristics of balansa clover which are important for annual seedling recruitment to maintain species persistence in a pasture.

3 Persistence and re-establishment

3.1 Introduction

The 'MaxClover' grazing experiment was established in 2002 at Lincoln University, New Zealand. The study compared production and botanical composition of binary pasture mixtures of cocksfoot (*Dactylis glomerata*) sown with annual and perennial legumes compared with perennial ryegrass and white clover (Brown *et al.*, 2006; Mills *et al.*, 2008a). In the establishment phase of the 'MaxClover' cocksfoot grazing experiment, balansa clover produced 2.3 t DM/ha ($P < 0.05$) compared with 1.1 t DM/ha for subterranean clover from 18 February to 8 September 2002 (Buckley unpublished). The balansa clover/cocksfoot pastures out-yielded subterranean clover/cocksfoot when there was only moderate grass competition. However, over six years, subterranean clover was more reliable at the 'MaxClover' site (Mills *et al.*, 2008a). This chapter focuses on the effect of different grazing management through the flowering period on balansa clover regeneration and proposes a management strategy to successfully integrate balansa clover into a pasture. The long term contribution of balansa clover dry matter to a pasture sward is addressed through the analysis of individual plot data, where plots deliberately received different spring/summer grazing management. Much of the dry matter production data for this chapter were collected by field technicians as part of the 'MaxClover' programme. However, the analyses and interpretation of that data are part of this PhD programme.

3.2 Materials and methods

3.2.1 Experiment 1

'Bolta' balansa clover (3.5 kg/ha) and 'Denmark' subterranean clover (10 kg/ha) were sown separately in a mixture with 'Vision' cocksfoot (4 kg/ha) on 18 February 2002 into four 0.05 ha fenced plots as 2 of the 6 mixtures sown as part of the larger 'MaxClover' dryland grazing experiment (Brown *et al.*, 2006) at Lincoln University. Two additional replicates of the six treatments were sown at the same site on 6 May 2003. The soil at the experimental site is a Templeton silt loam (Udic Ustochrept,

USDA Soil Taxonomy) (Watt and Burgham, 1992) with between 0.8 and 1.4 m to underlying gravels (Cox, 1978). Plant available water capacity (PAWC) for the top metre averages 142 mm/m (Moot *et al.*, 2008). All pasture types within the larger experiment were grazed to balance animal and pasture requirements. Balansa clover pastures were rotationally grazed from late August with 3 flocks grazing 2 paddocks each in 10-14 day rotations to achieve 6-800 kg/ha of residual dry matter. This changed to 2 flocks grazing 3 paddocks at 15-18 day rotations in late September. Each balansa clover pasture was closed in spring to re-seed. The timing and frequency of closure, and hence seed production, differed among the six balansa/ocksfoot plots (Table 3.1). The impact of this individual plot analysis forms the basis of this chapter. The effect of different spring spelling on subsequent balansa clover seedling recruitment, herbage productivity and botanical composition were measured within an exclosure cage. Botanical composition was determined from hand-sorted sub-samples. Caged areas (1 m²) were mown to 30 mm height prior to cage placement and moved to a freshly mown area every 28 days. Subterranean clover pastures were measured in the same way.

3.2.1.1 Metabolisable energy

Metabolisable energy (MJ/kg DM) was calculated using near infrared spectroscopy (NIR) analysis from ground samples of the sown components following methods described by Corson *et al.* (1999) calibrated using paired samples. Green herbage samples were taken for analysis beginning November 2003, when sufficient material allowed processing. The dates and number of replicates harvested for metabolisable energy analysis are presented in Appendix 1.

3.2.1.2 Seed in soil cores

Five 100 mm diameter soil cores were taken at random to a depth of 40 mm from each of Plots 1, 3, 5 and 6 on 11 June 2008. Soil cores were washed through 0.5 and 2 mm sieves and balansa clover seeds counted. Plots 1, 3, 5 and 6 were chosen to identify any seed bank difference resulting from their contrasting closing date management (Table 3.1).

3.2.2 Statistical Analysis

Because of the lack of repetition of closing times, total annual dry matter production of each plot was analysed using a one way ANOVA with time and species as treatments. Botanical composition was analysed by ANOVA as a split plot design with sown legume as main plots, years as split plots and six replicates. Individual component content within years was analysed by ANOVA as a complete block design with the two annual legume treatments and six replicates. There was no replication of the closing date treatments.

Table 3.1 The day of last grazing (spring closing date (C)) for balansa clover pastures sown with cocksfoot in a grazing experiment on 18 Feb 2002 (Plots 1-4) or 6 May 2003 (Plots 5&6) at Lincoln University, Canterbury.

Year	Pasture Plot					
	1		2		3	
	C	O	C	O	C	O
2002	6-Sep	1-Jan	23-Sep	8-Jan	24-Oct	16-Jan
2003	28-Oct	23-Dec	3-Nov	23-Dec	7-Nov	29-Dec
2004	29-Oct	19-Dec	4-Oct	10-Dec	11-Oct	10-Dec
2005	NC	NC	NC	NC	NC	NC
2006	27-Oct	3-Jan	5-Nov	11-Jan	29-Oct	20-Jan
2007	NC	NC	NC	NC	NC	NC

Year	4		5		6	
	C	O	C	O	C	O
2002	10-Oct	10-Jan	-	-	-	-
2003	12-Nov	29-Dec	-	22-Dec	-	22-Dec
2004	15-Oct	15-Dec	NC	NC	25-Oct	15-Dec
2005	NC	NC	NC	NC	NC	NC
2006	22-Oct	30-Jan	25-Oct	26-Dec	8-Nov	19-Dec
2007	NC	NC	NC	NC	NC	NC

Note: C is closing date, O is opening grazing date when grazing recommenced. NC is no closing for flowering and reseeding. Plots 5 and 6 were first grazed on 22 December 2003. The interval between C and O indicates the length of spelling time for each plot. Years are calendar years.

3.2.3 Meteorological conditions

Rainfall, evapotranspiration and temperature data were taken from Broadfields Meteorological Station, 2 km north of the experimental site, Canterbury, New Zealand. Long term means presented are the mean of data from 1975 – 1991.

3.2.3.1 Rainfall and evapotranspiration

Annual rainfall during the experimental period averaged 599 mm and ranged from 411 mm to 853 mm, with five years below and one year above the long term mean (LTM) (Figure 3.1). The LTM rainfall is 680 mm/year and is evenly distributed (50-70 mm/month). The experimental site was not irrigated at any time.

The moisture lost to evapotranspiration (Penman) (PET) during the experimental period averaged 1047 mm/year and ranged from 1008 mm in 2005 to 1091 mm in 2003, with 2 years below and 4 years above the LTM of 1033 mm/year. The average potential soil moisture deficit (rainfall minus PET) for the experimental period was therefore 448 mm/year.

For analysis of pasture growth rate, moisture was considered non-limiting when the soil water deficit was estimated to be below the critical limiting soil water deficit for this soil (78 mm) as determined by Mills *et al.* (2006). These authors compared pasture growth rate of an irrigated and non-irrigated pasture with known soil water status. Because the actual extraction data were not available from the site, the soil water deficit was estimated from rainfall and PET data from the Broadfields meteorological station, 2 km north of the experimental site.

3.2.3.2 Temperature and solar radiation

The mean daily air temperature followed a similar pattern each season, ranging from 7-8 °C in June-August to 15-17 °C in February (Figure 3.2). The mean daily 100 mm 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/m²/day in June/July to a maximum of ~25 MJ/m²/day in December.

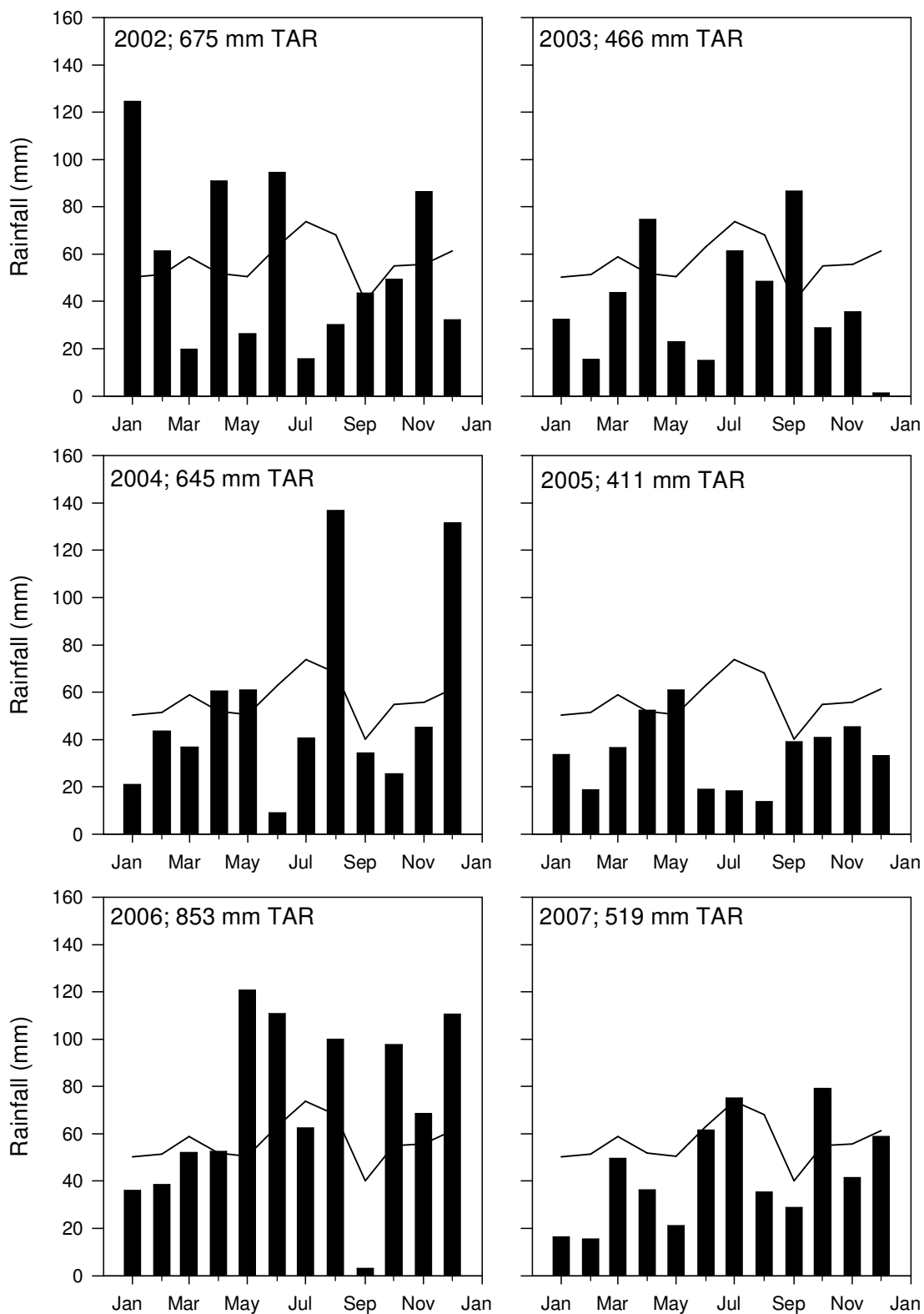


Figure 3.1 Monthly (■), long term mean (-) and total annual (TAR) rainfall (mm) from Broadfields Meteorological Station, 2 km north of the experimental site. Long term mean data from 1975-1991.

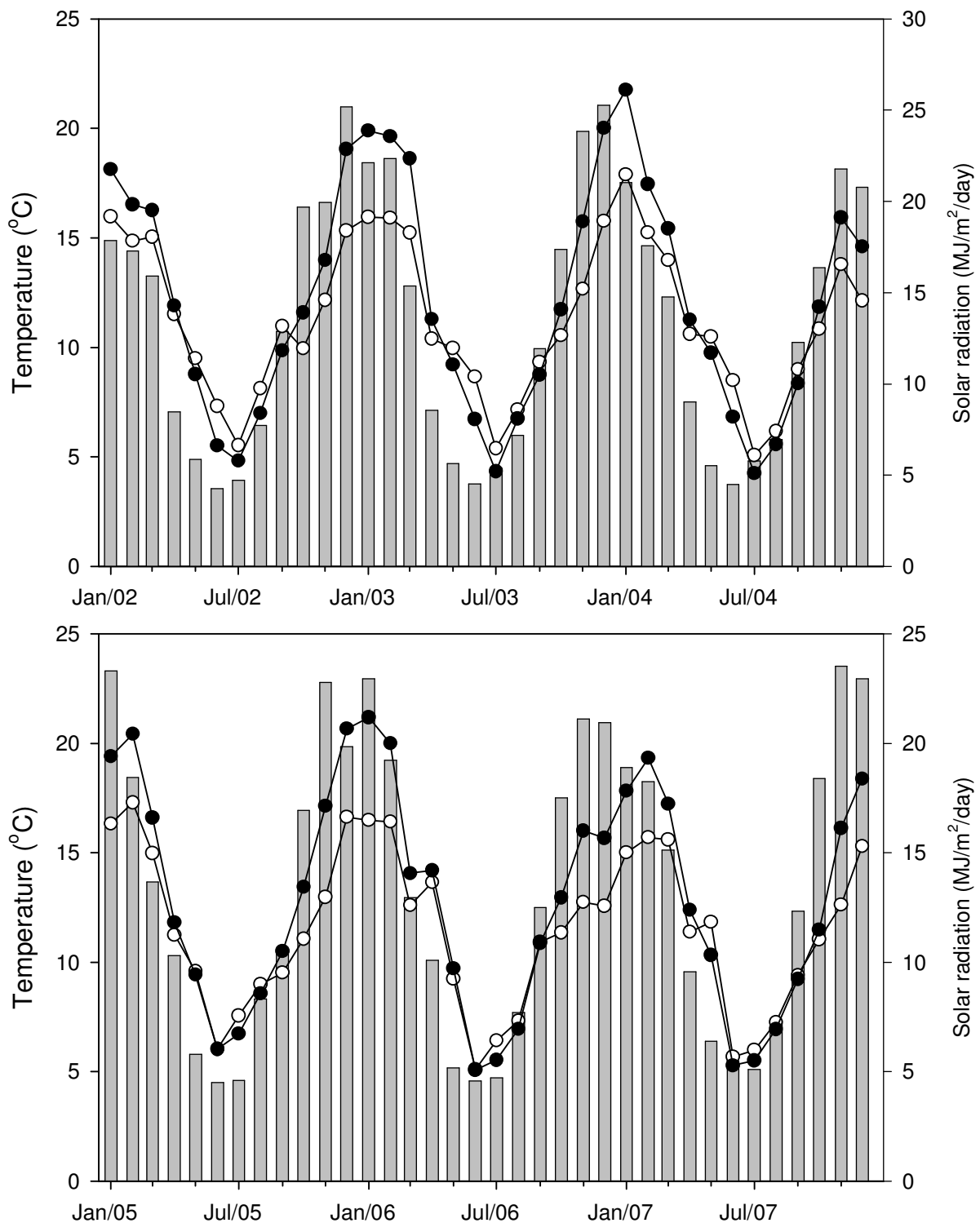


Figure 3.2 Monthly mean daily air (○) and 100 mm soil (●) temperatures, and mean daily solar radiation (PAR) (bars) per month from 1 January 2002 to 31 December 2007. Data are from Broadfields Meteorological Station, 2 km north of the experimental site, Canterbury, New Zealand.

3.3 Results

3.3.1 Balansa clover/ cocksfoot production and composition

Over the first six years, total dry matter (DM) production from the balansa clover/ cocksfoot pasture averaged 8.9 t DM/ha (Table 3.2). Total annual DM production was highest ($P=0.049$) in the second year with an average of 10.9 t DM/ha (Table 3.2). The highest ($P=0.004$) annual balansa clover content was in years 1, 2 and 4 and averaged 2.3 t DM/ha. The lowest annual balansa clover production was 0.4 t DM/ha recorded in year 5. In the following year, balansa clover produced 1.1 t DM/ha. Weeds, volunteer white clover and other grass dry matter increased over time ($P<0.001$) from 0.3 t DM/ha in year 1 to 2.1 t DM/ha in year 6.

Balansa contributed an average of 20.7% to the total annual dry matter production over 6 years across all plots. Total balansa content decreased from 31 to 6% from year 1 to year 5 (Table 3.2).

Table 3.2 Dry matter yield (t/ha) and botanical composition (%) of 'Bolta' balansa clover/ cocksfoot pastures sown at Lincoln University, Canterbury, New Zealand.

Production year	Total (t/ha)	Balansa (t/ha)	Balansa (%)	Cocksfoot (t/ha)	W/O/VWC (t/ha)	Dead (t/ha)
1	7.8 _b	2.4 _a	31.7	4.8 _b	0.3 _c	0.3 _b
2	10.9 _a	2.6 _a	26.7	6.9 _a	0.9 _{bc}	0.5 _{ab}
3	8.9 _b	1.3 _{bc}	23.3	6.5 _a	0.6 _c	0.5 _{ab}
4	8.9 _b	1.9 _{ab}	21.6	4.9 _b	1.4 _b	0.7 _a
5	8.2 _b	0.4 _c	6.2	6.0 _{ab}	1.1 _b	0.7 _a
6	8.8 _b	1.1 _{bc}	15.2	5.2 _b	2.1 _a	0.4 _b
Mean of the six years	8.9	1.6	20.7	5.7	1.1	0.5
P value	0.049	0.004	N.S.	0.001	<.001	0.007

Note: Where P values are ≤ 0.05 , the letters within columns represent mean separation by 5% LSD. W/O/VWC is weeds, other grasses and volunteer white clover. N.S. is not significant. Production years are from 1 July to 30 June, or as close as practical depending on actual harvest dates.

3.3.2 Sub clover dry matter production and botanical composition

Over the first six years, total dry matter production in the subterranean clover/cocksfoot pasture ranged from 8.2 t DM/ha to 13.3 t DM/ha (Table 3.3). Subterranean clover content also fluctuated through the recording period from 1.6 t DM/ha to 4.2 t DM/ha. Subterranean clover production was also lowest in the 5th year, but recovered in year 6 to produce 3.1 t DM/ha. Weeds, volunteer white clover and other grasses all increased ($P < 0.001$) with time, from 0.4 t DM/ha in year 1 to 2.3 t DM/ha in year 6.

Table 3.3 Dry matter yield (t/ha) and botanical composition (%) of 'Denmark' subterranean clover/cocksfoot pasture sown at Lincoln University, Canterbury, New Zealand.

Production year	Total (t/ha)	Sub. clover (t/ha)	Sub. clover (%)	Cocksfoot (t/ha)	W/O/VWC (t/ha)	Dead (t/ha)
1	10.5 _{bc}	4.2 _a	40.0	5.7 _{bc}	0.4 _b	0.2 _b
2	12.0 _{ab}	2.3 _{bc}	19.0	8.2 _a	0.7 _b	0.7 _a
3	9.0 _{cd}	2.1 _{bc}	23.0	5.7 _b	0.8 _b	0.5 _{ab}
4	13.3 _a	4.1 _a	30.9	6.6 _{ab}	1.8 _a	0.8 _a
5	8.2 _d	1.6 _c	19.1	4.4 _{cd}	1.7 _a	0.5 _{ab}
6	9.9 _{cd}	3.1 _b	31.0	3.9 _d	2.3 _a	0.6 _{ab}
Mean of the six years	10.5	2.9	27.2	5.8	1.3	0.5
P value	<.001	<.001	N.S.	<.001	<.001	0.035

Note: Where P values are ≤ 0.05 , the letters within columns represent mean separation by 5% LSD. W/O/VWC is weeds, other grasses and volunteer white clover. Sub is subterranean clover. N.S. is not significant. Production years are from 1 July to 30 June, or as close as practical depending on actual harvest dates.

3.3.3 Plot analysis

3.3.3.1 Balansa clover

Closing dates for each plot were given in Table 3.1. Of the four plots sown in 2002, Plot 1 had the earliest closing date (6 September) in the establishment season. It subsequently produced the highest ($P = 0.006$) (Section 3.2.2) total dry matter and clover dry matter over the six years of the four balansa clover pastures. Total herbage production of this plot averaged 9.3 t DM/ha/year (Figure 3.3), with between 2.2 and 4.3 t DM/ha/year of balansa clover over all years. In 2006, balansa clover dry

matter production in Plot 1 decreased to < 0.5 t/ha but recovered to > 3 t/ha in 2007 after an October closing in 2006 (Table 3.1). Of the 2002 sown plots, balansa clover dry matter production only recovered in 2007 in Plot 1 (Figure 3.3). The other three plots produced less than 0.7 t balansa clover DM/ha in 2007.

Plot 5 was sown in 2003 and had no grazing and full flowering in 2003, but was managed to prevent seed production in 2004 or 2005 (Table 3.1). Clover dry matter production in Plot 5 decreased from ~2.5 t/ha/year in 2004 and 2005 to < 0.5 t/ha in 2006 and 2007 (Figure 3.3). In contrast, Plot 6, which also had no grazing and full flowering in 2003 was allowed to reseed in 2004 and 2006 and recovered from <1.0 t/ha of clover content in 2006 to almost 3 t/ha of balansa in 2007.

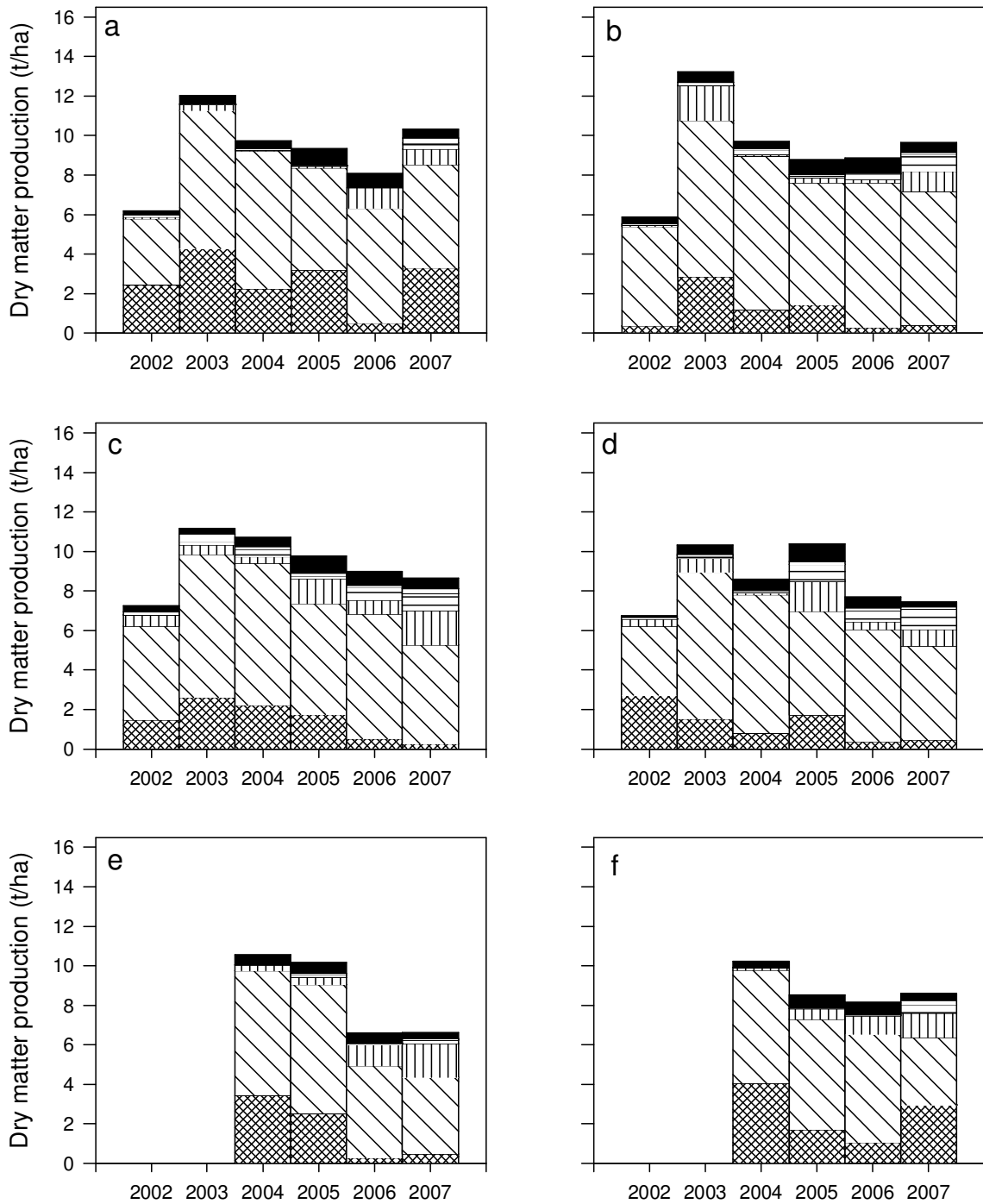


Figure 3.3 Annual yields (t/ha) of pasture components from balansa clover/cocksfoot pastures from six individually managed plots sown in the 'MaxClover' experiment at Lincoln University, Canterbury, New Zealand. (⊗) balansa clover, (▨) Cocksfoot, (▧) Other grasses, (▩) Volunteer white clover, (□) weeds and (■) dead. (a) Plot 1, (b) Plot 2, (c) Plot 3, (d) Plot 4, (e) Plot 5, (f) Plot 6.

Note: Plots 1-4 were sown in 2002. Plots 5-6 were sown in 2003 and not recorded until 2004.

3.3.3.2 Subterranean clover

By comparison, the subterranean clover dry matter production in adjacent plots was always greater than in the balansa clover pastures. The dry matter production in the two comparative subterranean clover plots also decreased in 2006 to 1.4 t clover DM/ha but both plots recovered and produced more than 3.4 t clover DM/ha in 2007 (Table 3.3). Mean subterranean clover production in the cocksfoot pasture during years 2-5 was ~3.8 t DM/ha/year with total herbage production of ~7.3 t DM/ha.

3.3.3.3 Seed in soil cores

On 11 June 2008 there were more ($P=0.002$) balansa clover seeds present in cores taken from Plot 1 than from either Plots 3, 5 or 6 (Table 3.4). Cores taken from Plot 6 had ~60% more seeds than Plot 5, but this was not significantly different ($P>0.05$).

Table 3.4 The number of balansa clover seeds in a 100 mm diameter soil core sampled to a depth of 40 mm on 11 June 2008 from balansa clover/cocksfoot binary pasture mixtures sown in the 'MaxClover' experiment at Lincoln University, New Zealand.

Plot	1	3	5	6
Seeds/core	88 ^a	16.6 ^b	24.4 ^b	40.8 ^b
Seeds (kg/ha)	132	25	37	61

Note: $P=0.002$, means separation by LSD $\alpha=0.05$, with similar letters used to indicate no significant difference. Kg/ha analysis assumes 1.28M cores/ha and a seed weight of 1.17mg.

3.3.4 Metabolisable energy

The mean metabolisable energy (ME) content of balansa clover from 11 December 2003 to 27 November 2007 across all plots was 11.8 MJ/kg DM. Balansa clover and white clover had a higher ($P<0.001$) mean ME content than subterranean clover during that period. All these clovers had the same mean ME between August and October of 12.2 MJ/kg DM.

3.4 Discussion

Closing date of the balansa clover plots in this grazed system was deliberately varied to create differences in seed set in the establishment and subsequent years. As a

consequence, production and persistence of balansa clover based pastures in some plots were compromised as the impact of grazing management was assessed. The differences in individual plot management within the grazing experiment offer insights into how the seed bank, as the source of seed for re-establishment, can be managed to maintain balansa clover in a pasture.

The difference between recovery and failure of balansa clover following very low production and seeding opportunities in 2006 was related to the closing date of the plots in previous years, particularly the establishment season. Plot 1 benefited from the earliest closing date (Table 3.1) in the establishment year (2002) and closing for limited seed inputs in the following two seasons. In Plot 1, balansa clover contributed the highest proportion of clover in Year 4 with 3.2 t DM/ha (34% total DM) and Year 6 with 3.3 t DM/ha (32% total DM) (Figure 3.3). The management of Plot 6, sown in 2003, also produced sufficient seed for balansa clover to successfully re-establish and contribute 2.9 t clover DM/ha three years later in 2007 (Figure 3.3).

Plot 6 had no grazing in 2003 and then limited flowering and seeding in 2004. Plot 5 was managed to contrast with Plot 6 by not allowing any seed set in 2004 (Table 3.1). As a result, Plot 5 produced sufficient seed in the establishment year (2003) for balansa clover to successfully re-establish for the next two years. However, the single seed set in 2003 was insufficient for Plot 5 to produce more than 0.5 t DM/ha in the third and fourth years (Figure 3.3).

3.4.1 Seed in soil cores

On 11 June 2008, six years after initial sowing, Plots 1, 3, 5 and 6 each had 25 kg/ha or more balansa clover seed within the top 40 mm (Table 3.4). Estimates of seed production in Plot 1 in the establishment year were >1000 kg/ha (Pers. Comm., Richard Lucas, Lincoln University, New Zealand, 2009). The late closing dates of Plot 3 of mid October to early November (Table 3.1) were able to provide at least 25 kg/ha of balansa clover seed, or 21.5 kg/ha more seed than was sown. In Plot 3, more than 25 kg/ha of seed was unable provide sufficient seed, be it through hardseed softening or sheer weight of numbers, to overcome the competition from established pasture components at the break of the season in autumn. This further

highlights the need to maintain control of the summer herbage to promote balansa clover re-establishment. Dear *et al.* (2007) showed that there was also a strong relationship between the number seedlings and the size of the seed bank. Subterranean clover required a minimum contribution of 200 kg/ha/year seed to maintain persistence in a pasture with perennial grasses.

These contrasting results suggest that the potential exists for balansa clover to persist in a grazed pasture with cocksfoot for at least three years after each flowering. The plot grazing management records have identified the need to allow prolific seeding in the establishment year as a key component of balansa clover dry matter productivity in subsequent years. However, a large seed set alone (>400 kg/ha) is not a guarantee of success. Summer and autumn grazing management must be targeted to promote balansa clover re-establishment (Section 3.4.2).

The failure in balansa clover production in Plots 2, 3, 4 and 5 in 2007 resulted from a dry autumn in 2006 which limited annual clover regeneration in all annual clover plots (Mills *et al.* 2008, Table 3.2 and Table 3.3). Between 6 February 2006 and 24 April 2006 only 77 mm of rain fell and Penman evapotranspiration totalled 263 mm. Observations indicated seedlings which emerged from rainfall in February died, due to insufficient soil moisture, before the subsequent rain in late April. This false break led to low annual legume populations and low spring production in these pastures. In a demographic model of clover persistence, Jansen and Ison (1996) highlighted the sensitivity of balansa clover to low seed production and the risk of false strike. Subterranean clover seedling regeneration has also been shown to be related to the number of seeds stored in the seed bank (Dear *et al.*, 2007). Both these factors appear to have contributed to the annual clover failures in 2006, when both balansa and subterranean clover had decreased dry matter yields (Table 3.2 and Table 3.3).

In 2005, when all six balansa clover based pasture plots were grazed throughout spring, ewe hogget live-weight gain was comparable between balansa clover and subterranean clover based pastures (Brown *et al.*, 2006; Mills *et al.*, 2008b). These data suggest that if all plots were managed in line with Plot 1, balansa clover based pastures could produce animal live-weight gains similar to those from subterranean

clover based pastures. This interpretation is in line with other balansa clover recruitment studies published in central west New South Wales (Jansen and Ison, 1996; Jansen *et al.*, 1996). From their simulation work they concluded that for pure sown balansa clover stands, successful re-establishment each year depended on the amount of seed set. They showed seed set between 400 and 1200 kg/ha was required each year to overcome the drain on the seed bank from predators, false breaks and hardseededness. However, the data here refutes this in our environment. In a temperate climate, where the breakdown of hardseed is slower than in New South Wales, balansa clover was able to regenerate from hardseed stored in the seed bank for at least three years from an estimated >1000 kg/ha seed in the establishment year (Plot 1). The breakdown of balansa clover hardseed in the field at Lincoln University, New Zealand is discussed in Section 6.4.3.

3.4.2 Grazing management

There are two dominant farm management practices in New Zealand dryland farm systems which act against balansa clover re-establishment. The first involves having animals set-stocked on paddocks throughout the spring period during lambing and lactation. In contrast, on cash cropping farms in Australia, where the commercial cultivars of balansa clover were developed, annual legumes are used between cropping cycles and as feed for merino sheep for wool production (Dear, 2003). In the Australian system, the annual legume is allowed to set the maximum amount of seed each season by removing grazing animals before flowering begins. Once the legume has produced viable seed, animals are returned to the paddock to graze the standing herbage to the ground.

This system ensures the annual species produces a large amount of seed which then has low competition at establishment in the following season. Grazing pressure is then maintained throughout the year until animals are removed again at the time of flowering. Jansen and Ison (1995) showed grazing in summer did not reduce the amount of balansa clover seed in the seed bank because most seeds had fallen from the dry inflorescences out of easy access to grazing animals. The physiologically mature balansa clover seeds which are eaten in summer are likely to pass through the gut and remain viable. Carter *et al.* (1989) showed seeds similar in size to

balansa clover (e.g. Persian clover) passed through sheep with a low level of death or destruction. Larger seeds like those of subterranean clover were reduced to 1% survival (Carter, 1983). For balansa clover, this could then act as a method of seed distribution on farm.

In contrast to that seen in Australia, the dominant dryland grazed farming system in New Zealand is sheep and beef production (Matthews *et al.*, 1999). In most New Zealand operations both sheep and cattle are flushed in autumn (March/April) and produce offspring in spring (September) to match the increase in feed supply during the periods of high soil moisture and favourable temperatures. The need to have ewes set stocked at lambing in paddocks of high quality herbage creates a problem for seed production in top flowering annual legumes, like balansa clover. Alternative stock policies are discussed in more depth in the General Discussion (Chapter 7).

3.4.2.1 Summer grazing management

The second practice is related to the reduced summer grazing pressure which is typical in New Zealand. Annual and perennial grass species lose nutritive quality and have low grazing preference when they develop reproductive structures in the spring/summer period (Lambert and Litherland, 2000). Because of this, reproductive growth is usually coupled with a decline in animal performance of stock on hand. Lambs finished in November/December and old ewes after weaning are sold in anticipation of the forthcoming drought. By decreasing stock numbers the grazing pressure on farm is reduced for the summer period. The subsequent inability to create bare ground in a paddock dramatically limits balansa clover recovery (Dear and Coombes, 1992). The failure of balansa clover to re-populate beyond the establishment year in the 'MaxClover' experiment (Figure 3.3) was also seen in an Australian mixed pasture experiment when grazing pressure in summer/autumn was too low in the summer (Dear *et al.*, 2002). The worst-case scenario for balansa clover re-establishment in autumn is, therefore, a wet summer, during which the grass recovers faster than it can be grazed with the limited stock available, followed by a dry autumn.

3.4.2.2 Establishment year

With these aspects in mind, when early (Jan/Feb) rains initiate germination and growth, pastures should be grazed to control emerged weeds once 'Bolta' balansa clover seedlings are not easily pulled from the soil by the finger and thumb. Grazing pressure should be increased in March and April with the requirement for flushing (Dear *et al.*, 2002) and grazing should continue through winter. Australian recommendations for set-stocking in winter (Snowball, 1994) should be substituted for rotational grazing in New Zealand, where paddock sizes are generally smaller and legumes, which are preferentially grazed, are given time to recover (Edwards *et al.*, 1993). As the rate of pasture growth increases in spring (Brown *et al.*, 2006; Mills *et al.*, 2008a), ewes and lambs or young dairy stock etc. could be brought in to graze the herbage. The timing of when animals should be removed from the pasture depends on the time of flowering (Section 5.3.1), but in Plot 1 of the 'MaxClover' experiment, animals were removed completely by mid-September to allow 'Bolta' to flower and set seed.

The pasture would then be grazed throughout summer from late-November, when seed had shed, to maintain control of the more aggressive components to target ~700 kg DM/ha, with 20-30% of the soil surface bare, in February (Leigh *et al.*, 1995; Dear *et al.*, 2002). In the establishment year, the standing herbage could be cut for a hay crop once the balansa clover seed has become mature. The balansa clover seed-laden hay crop could then be fed out over summer in other paddocks as a way of spreading the seed around the farm. The timing of seed maturation is addressed in Section 5.3.2.1. Whichever summer strategy is employed, herbage must be removed before the onset of autumn rains. With sufficient seed set, balansa clover will regenerate without the need to remove grazing animals in September for another four years.

3.4.2.3 Second and subsequent years

In the second and subsequent years, the balansa clover based pasture should be rotationally grazed throughout autumn and grazing pressure increased in late winter and early spring. Ewes and lambs can graze the pasture throughout the spring with ewes after weaning and/or cattle in summer to control pasture mass as required. The

same ~700 kg DM/ha pasture mass and 20-30% bare soil targets for February apply wherever annual clover persistence is desired. Balansa clover requires a large re-seeding event once in every 4-5 years to maintain the seed bank population. This might occur in favourable years when there is surplus feed available in other areas of the farm or as part of a general paddock rejuvenation policy. For example, the ability to make hay from plots in the field experiment would have helped to remove unwanted bulk.

3.4.3 Wet summers, grass growth and grazing management

Above average rainfall in the summer of 2004 and 2006 (Section 3.2.3.1) led to high cocksfoot summer production. Cocksfoot (Mills *et al.*, 2006) and other perennial grasses (Dear and Cocks, 1997) are able to recover rapidly from a period of drought-induced dormancy with late season rainfall. This meant when any rain fell on the 'MaxClover' site, cocksfoot was likely to have utilised available moisture to re-establish leaf area rapidly. In December 2004, 132 mm of rain fell. The increased grass growth led to shortened grazing rotations with 3 mobs of lambs grazing 2 plots each from 14 February in balansa clover/cocksfoot and subterranean clover/cocksfoot pastures. This measure was not sufficient to contain the increased grass growth and therefore compromised the balansa clover component. The 3 mobs of lambs continued grazing the balansa clover/cocksfoot pastures for 14 weeks, until early June, and for 7 weeks on the subterranean clover/cocksfoot pastures in an attempt to control the cocksfoot grass growth. The lambs were unable to exert sufficient pressure on the plots to create bare soil for emerging annual clover seedlings in autumn 2005 (Pers. Comm., Malcolm Smith, head technician working with the 'MaxClover' experiment, Lincoln University, New Zealand, 2008), therefore seedling recruitment was compromised in the following season. Shifting animals daily between plots to non-selectively graze or using a different class of animal (dry ewes, for example) may assist with controlling excess herbage production.

This inability to control the grass with grazing animals in wet summers highlights one of the key areas of balansa clover based pasture management which was identified at the experimental site. Cool season annual clovers require bare ground to emerge into during autumn (Dear, 2003). Without sufficient grazing pressure in the summer,

balansa clover has also failed to re-establish in grazed experiments in Australia (Dear *et al.*, 2002). Legumes must compete with perennial species for limited light, moisture and nutrients at establishment (Dear *et al.*, 2007).

3.4.3.1 Re-establishment

Decreased perennial grass sowing rates and increased row spacings may help to increase the light penetration to the balansa clover (Wolfe and Southwood, 1980). Once the pasture is established, top-working the paddock with spring-tyne harrows to open the sward and the addition of superphosphate may promote balansa clover establishment where grazing cannot control green leaf area of perennials coming into autumn. This method is referred to as 'top-worked greenfeed' (Pers. Comm., Warwick Scott, Lincoln University, New Zealand, 2009).

The balansa clover cultivars which are currently available have relatively early flowering dates compared with subterranean clover cultivars (Wurst *et al.*, 2004). Balansa clover/cocksfoot may perform more favourably compared with subterranean clover or white clover in environments where the available moisture is used by the end of October, e.g. a very stony Lismore soil (PAWC of 56 mm/m (from lucerne to 2.3 m)) (Moot *et al.*, 2008) at Ashley Dene, Canterbury, New Zealand.

The relatively high level of extractable soil moisture at the 'MaxClover' experimental site meant the benefit of an early flowering annual clover cultivar, like 'Bolta' balansa, may have been overshadowed by later flowering annual clovers (e.g. 'Denmark' subterranean clover) and summer active perennial clovers. Selecting the best legume species for the environment therefore requires consideration of both dry matter production within the average growing season and the ability to maintain persistence (Norman *et al.*, 2005).

Subterranean clover in comparable conditions in adjacent plots was able to produce seed, germinate, emerge and survive to become plants contributed >3 t DM/ha each year (Table 3.3). Thus, differences in seedling characteristics, relative growth rate and competitiveness of balansa clover and subterranean clover may also contribute to the difference in dry matter contribution to mixed pastures. Given balansa clover

successfully re-established and contributed dry matter to a mixed pasture in two out of the six plots, it is possible it could be integrated into a dryland pasture system. However, the failure to re-establish in the other four plots means the mechanisms for success need to be investigated.

The following chapters of this thesis describe studies designed to quantify the physiological characteristics of balansa clover as a seedling.

3.5 Conclusions

This analysis of a long term, grazed dryland experiment has shown balansa clover can persist when sown with cocksfoot, producing more than 3 t DM/ha of clover five years after establishment. This analysis has also shown that successful regeneration was dependent on grazing management, particularly in the establishment year. Specific conclusions are:

- Balansa clover closed on 6 September for flowering and seed production in the establishment season (Plot 1) maintained satisfactory dry matter contribution in a cocksfoot based pasture for 3 years. Because of this, closing the pasture for flowering each year was not necessary. In June 2008, six years after sowing, Plot 1 contained 132 kg/ha balansa clover seed in the top 40 mm of soil.
- Adding to the balansa clover seed bank in years that are favourable to seed set enabled persistence beyond the third year. Plot 6 pasture produced seeds in the 2nd and 3rd years after sowing and was able to recover to produce 3.3 t DM/ha of clover in the 6th year after little seed was added to the seed bank in years 4 and 5. In June 2008, Plot 6 contained 61 kg/ha balansa clover seed in the top 40 mm of soil.
- Grazing management and stock policies must be targeted towards suppressing the grass component of the sward to promote annual clover emergence, establishment and productivity.

4 Vegetative development

4.1 Introduction

The results in Chapter 3 showed balansa clover successfully re-established and contributed dry matter on a yearly basis under specific management regimes. The next step in successful integration of balansa clover into a pasture system is to develop an understanding of the physiology of the plant. Specifically, this chapter describes the rate of balansa clover seedling development in comparison with other species sown in dryland areas of New Zealand.

To quantify the vegetative stages of development of balansa clover the timing of emergence, first leaf appearance, interval between successive mainstem leaves (phyllochron) and axillary leaf appearance are examined as key development stages. These components contribute to a plant's ability to compete for resources, especially light, by combining with leaf area to drive canopy expansion (Hay and Walker, 1989). They make up the major components of seedling development and establishment. For example, Caucasian clover seedlings are poor at establishing when sown with most other species because of the high thermal time requirement for axillary leaf appearance (Section 2.7).

Jamieson *et al.* (1995b) showed the relationship between temperature and the rate of leaf appearance in wheat depended on the location of the apical meristem, as the point of temperature perception (Section 2.6.1). For example, the phyllochron for 'Demand' white clover has been reported to be 126 °Cd ($T_{\text{base}} = 1\text{ °C}$) when the temperature was recorded at the soil surface (Black *et al.*, 2003b) and 94 °Cd ($T_{\text{base}} = 0\text{ °C}$) when recorded at Stevenson screen height (~1200 mm above the soil) (Black *et al.*, 2003a, 2006a). The contrast in the phyllochron reported by the same authors for 'Demand' white clover suggests there were differences between the temperatures recorded and the temperatures perceived by the plant in at least one of the two instances. An aim of this study then was to define the most appropriate temperature measurement location for each vegetative development phase compared with the most commonly available temperature: the Stevenson screen.

The objective of the experiments described was to quantify the time between seedling phenophases, namely sowing to emergence, the phyllochron and the timing of axillary leaf appearance and then to relate these to the underlying environmental driver which is temperature (Section 2.3).

4.2 Materials and methods

4.2.1 Experiment 2: Field

In Experiment 2, two cultivars of balansa clover sown in the field to quantify the rate of emergence, timing of first leaf appearance, the phyllochron and the timing of axillary leaf appearance during the establishment season. Different sowing dates were used to create temperature differences for thermal time analysis. Experiment 2 was continued to include measurements of reproductive development and seed recruitment and these are described in Chapters 5 and 6. 'Bolta' and 'Frontier' were chosen as they were the only two cultivars available in New Zealand.

4.2.1.1 Site description

The experimental area was located in Iversen Field, block 4, at Lincoln University, Canterbury, New Zealand (43° 38'S, 172° 28'E, 11 m a.m.s.l). The soil is classified as a Templeton silt loam (*Udic Ustochrept*, USDA Soil Taxonomy) with 1.8 – 3.5 m of fine textured material overlying gravels (Cox, 1978). Templeton soils are imperfectly drained and display a strong mottling which indicates periods of water logging. A soil test on 9 May 2004 gave the following results (Table 4.1). On 13 July 2005, sulphur superphosphate (8.7% P, 14.7% S, 19% Ca) at 200 kg/ha and urea (46% N) at 100 kg/ha were applied. This soil type was employed due to its accessibility for the author to facilitate frequent inspections and measurements required by the experiment. A Templeton silt loam soil is not a recommended soil type for studies on annual pasture legumes.

Table 4.1 Soil test (0-150 mm) results for the Iversen 4 block at Lincoln University, Canterbury, New Zealand on 9 July 2005.

Analysis	Result	Recommended range
pH (H ₂ O)	5.4	5.5+
Olsen P (µg/ml)	13	20-30
Ca ⁺⁺ MAF (unitless)	5	4-10
Mg ⁺⁺ MAF (unitless)	21	8-10
K ⁺ MAF (unitless)	10	5-8
SO ₄ -S (µg/g)	6	N/A

Note: MAF = samples were analysed using Ministry of Agriculture and Fisheries (MAF) quick test procedures.

4.2.1.2 Experimental design and sowing method

This field experiment (2) was a split-plot factorial in a randomised complete block design with three replicates and 48 plots. Main plots were 8 sowing dates (Table 4.2) and two balansa clover cultivars ('Bolta' and 'Frontier') as subplots. Before the first sowing, the experimental area was cultivated using conventional methods to produce a firm, fine seedbed. Conventional methods were also used to return the soil to a similar condition prior to each additional sowing through the 15 month sowing period.

Bare seed without inoculum (sourced from Agricom Ltd., Ashburton, New Zealand) was sown pure at 6 kg viable seed/ha based on germination tests. The plots were 2.1 m x 6.0 m and drilled at 150 mm row spacings at a target depth of ≤10 mm using an Øyjord cone seeder with chain harrows following. The plots were then Cambridge rolled. Both 'Bolta' and 'Frontier' balansa clover appeared to nodulate effectively with the resident rhizobia.

4.2.1.3 Management

All plots received 35 mm of irrigation water on 13 and 20 April 2006 to ensure establishment and survival of seedlings. All sown plots were sprayed on 28 October 2006 with Pulsar (200 g/l bentazone and 200 g/l MCPB) at 5 l/ha and Gallant (100 g/L haloxyfop) at 1.5 l/ha for the control of broadleaf and grass weeds (particularly *Poa annua*), respectively. Plots were not grazed.

Table 4.2 The eight sowing dates (SD) for Experiment 2 sown at Lincoln University, Canterbury, New Zealand.

SD1	14 Oct 2005
SD2	1 Dec 2005
SD3	16 Jan 2006
SD4	8 Mar 2006
SD5	27 Apr 2006
SD6	3 Jul 2006
SD7	30 Aug 2006
SD8	5 Feb 2007

4.2.1.4 Measurements

4.2.1.4.1 Soil and air temperature

Prior to sowing, four temperature sensors (Thermistors KTY-110) were placed at the experimental site. Two were placed within the soil at a depth of 10 mm, one at 100 mm and one at 1200 mm above the soil surface, in a central location within 3 m of all replicates. Temperatures were recorded every 5 minutes and integrated and logged every hour with a HOBO data logger (Onset Computer Corporation) to define the daily maximum and minimum temperatures for thermal time calculations.

4.2.1.4.2 Emergence

Emergence was defined as when both cotyledons had expanded. Emergence was recorded in four of eight sowing dates (SD4, SD5, SD6 and SD8). Two adjacent one metre sections of non-border rows were marked within each plot after sowing. The number of emerged seedlings was recorded at least twice weekly until seedlings ceased to emerge. The number of days after sowing to reach 50% of the final emergence (DAS50) was determined from a fitted generalised logistic curve that estimated the sigmoid shape of mean data for each plot (Equation 4.1). The curve was fitted using Genstat Tenth Edition (10.1.0.72) (Lawes Agricultural Trust).

Equation 4.1
$$y = a + C / (-B^{(X-M)})$$

Where a is the lower asymptote, C is the upper asymptote, B depends on the values of $y(0)$ and M is the time of maximum growth.

Thermal time analysis for emergence was performed using measured air and soil temperatures. The method chosen for each successive development stage was based on the highest coefficient of determination (R^2) for least squares regression of temperature against the rate of development. Where two results were similar, the physical proximity to the apical meristem (as the location of temperature perception in the plant) was chosen (Peacock, 1975). Because of this, 10 mm soil temperature was used in the analysis of emergence. Thermal time accrued each day was calculated as the mean of eight temperature values. The eight values were generated from the maximum (MaxT) and minimum (MinT) daily temperatures using a function to model the sigmoid shape of temperature change through a day (Jones and Kiniry, 1986). Each of the values was calculated and then had base temperature subtracted before being averaged to give a single value of thermal time for each day. The model was of the form:

Equation 4.2
$$0.92105 + 0.114 \times I - 0.0703 \times I^2 + 0.0053 \times I^3$$

Where $I = 1$ through to 8.

4.2.1.4.3 Leaf appearance

The number of leaves (unifoliate and trifoliate) was counted on six marked plants per plot at 3-5 day intervals until plants had a mean of 50 leaves or the first signs of leaf senescence. Leaves were considered to have emerged when the petiole was visible (Carlson, 1966). The timing of the first trifoliate leaf to appear on the mainstem was not counted in the field because competition from other seedlings made marking plants of consistent size and development stage difficult until they had two or more trifoliate leaves.

The leaf appearance rate (leaves/day) was determined by least squares regression analysis of the number of mainstem leaves against the number of days after

emergence. The mainstem leaf appearance interval (phyllochron) was determined by least squares regression analysis of the rate of leaf appearance against the mean daily thermal time accumulations for each period.

Thermal time (Equation 4.2) analysis for the determination of phyllochron was based on air temperature (1200 mm, Stevenson screen height) as the best approximation of the temperature that the plant was experiencing, because the shoot apical meristem of balansa clover is above the soil surface at emergence. Analysis was also performed using 10 mm soil and soil surface temperatures and combinations of different temperature sensing locations at different stages of development (Jamieson *et al.*, 1995b), but, based on R^2 , none were better than air temperature throughout the development of the plant. The reciprocal of phyllochron ($^{\circ}\text{Cd}/\text{leaf}^{-1}$) was used to allow linear relationships by least squares regression to be fitted (Section 4.2.3).

4.2.1.4.4 Axillary leaf appearance

The total number of leaves, both mainstem and axillary (those leaves that appear in the axil of a mainstem leaf) were also counted from five sowing dates (SD2, 3, 4, 5, and 6). Axillary leaves were considered to have emerged when the petiole was visible. The appearance of axillary leaves was determined to be the point when the number of total leaves exceeded the number of mainstem leaves by one. The number of total leaves was modelled using an exponential growth curve, of the form:

Equation 4.3 $y = d^{(nx)}$

Where d is constant and n is a curve shaping coefficient.

The rate axillary leaves appeared was modelled against the mean daily thermal time accumulations for each period using air temperature:

Equation 4.4 $y = e + g \times (r^x)$

Where e is the y intercept, g is a constant and r is a curve shaping coefficient.

4.2.2 Experiments 3-7: Growth room

In Experiments 3-7, 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover (*Trifolium resupinatum*) were sown in pots in growth rooms to quantify the rate of emergence, timing of first leaf appearance, phyllochron and the timing of axillary leaf appearance. Experiments 3-5 were also used to quantify the leaf size and the relationship between above ground plant weight and leaf area through specific leaf weights. The growth room temperature was changed in each experiment to allow thermal time analysis to be integrated across all experiments.

4.2.2.1 Sowing and design

Experiments 3-7 used a randomised complete block design with three species and four replicates. 'Laser' Persian clover was chosen because it is a top flowering annual clover that may have potential in dryland pastures in New Zealand. Four replicates of 50 bare, hand scarified (legumes) seeds (sourced from Agricom, Ltd., Ashburton, New Zealand) of each species were sown into a bark and pumice potting mix with 1 g/L Osmocote Plus (15% N, 5% P, 11% K), trace elements and 1 g/L dolomite lime (11% Mg, 24% Ca) in individual 4.5 L plastic containers in a growth room (Convicon PGV36, Winnipeg, Canada). 'Frontier' balansa clover was washed to remove the lime based coatings applied by the supplier. 'Frontier' seeds were wetted while vigorously rubbed against steel mesh to remove the coatings. All seeds were placed on the potting mix with 10 mm of sieved (1 mm) potting mix added on top. Pots were watered by hand as required at 3-5 day intervals.

Pots were placed on a raised, slatted table 1.4 m high (top of pots) and were re-randomised within blocks, within the growth room every 14 days. Photoperiod was set for 8 hours light and 8 hours dark with 4 hour transitions. The chamber was lit with a combination of incandescent (Sylvania, 40 W) and 30 fluorescent (Sylvania, 6 x 115 W and 24 x 215 W) lamps. Temperature within the growth room was recorded using Thermistors KTY-110 temperature sensors and HOBO data logging equipment (Onset Computer Corporation), with two sensors at 10 mm depth of soil, one at the soil surface covered in foil and one shaded at ~1 m above ground level recording air temperature. Plants were hand thinned as required to reduce competition for moisture, nutrients and light, following the procedures described by Black *et al.*

(2002). The mean daily thermal time accumulation for each experiment is given in Table 4.3 and an example of a 24 hour period is given in Appendix 2.

Table 4.3 The experiment number and mean daily temperature within Conviron PGV36 growth room used for quantifying the seedling phenophases of a range of herbage species.

Experiment number	Mean daily temperature (°C)	
	Air	10 mm below soil surface
3	6.8	11.5
4	10.5	15.0
5	15.6	19.6
6	20.8	23.8
7	25.5	27.3

4.2.2.2 Measurements

In addition to rate of emergence, leaf appearance and axillary leaf appearance, individual plant weight and leaf area were recorded in Experiments 3, 4 and 5.

4.2.2.2.1 Plant weight and leaf area

Individual plant weight and leaf area were recorded once each in Experiment 3 and 4 and twice in Experiment 5. Plants were destructively harvested to ground level and leaf area recorded using a bench-top mounted leaf area meter (LI-COR model LI-3100, LI-COR Inc., Nebraska, USA). The leaf weight and leaf area were analysed by ANOVA (Genstat, Tenth Edition) using mean plant values within each pot as replicate data, once differences between curves had been determined by 95% confidence interval (CI, represented by the symbol †). Specific leaf weight was calculated as the above ground weight per unit leaf area (g/cm^2).

4.2.3 Analysis

To determine the cardinal temperatures: base temperature (T_{base}), optimum temperature (T_{opt}) and maximum temperature (T_{max}), different calculations were used. To calculate the thermal time (Tt) required for 75% of maximum germination and

T_{base} , least squares regression analysis was used for the positive linear portion of the line (Equation 2.1) where:

Equation 4.5 $Rate = b_0 + b_1x$

The regression coefficients were then be related to T_{base} and Tt (Equation 2.2), defined by Angus *et al.* (1981) as:

Equation 4.6 $T_{base} = -b_0 / b_1$

As germination rate deviated from the linear model, data were excluded from the analysis. This deviation indicated that the temperature experienced had moved above the optimum (T_{opt}) for that development process and that the rate of development was decreasing towards to T_{max} (Thompson, 1974; Angus *et al.*, 1981). A similar calculation was made with the linear decreasing portion of the relationship to determine the T_{max} . An iterative broken-stick regression analysis (Draper and Smith, 1998) was used to find the optimum temperature.

Where 95% confidence intervals included 0 °C for the x -axis intercept, Tt was re-calculated with $T_{base} = 0$ °C to allow direct comparison among species (Moot *et al.*, 2000).

The coefficient of determination presented in tables and figures for base (T_{base}) and optimum (T_{opt}) temperatures and thermal time (Tt) were calculated from two individual least-squares regression analyses using Genstat Tenth Edition (10.1.0.72) (Lawes Agricultural Trust). The first fitted line was formed using data points recorded between the minimum and maximum rate of development (T_{base} and T_{opt}). The coefficient of determination presented for Tt ($T_{base} = 0$ °C) was calculated from the linear regression from T_{base} and T_{opt} . The coefficient of determination presented for maximum temperature (T_{max}) was calculated from the second linear model, using data points recorded between T_{opt} and T_{max} . Where appropriate, T_{opt} were estimated from the intersection of the two linear equations. The 95% confidence interval (CI, represented by the symbol †) was calculated for the x -axis intercept, where the y -axis = 0.

Standard errors presented for final number of leaves were calculated using ANOVA for a randomised complete block analysis where species was the treatment and replicates were blocks. The maximum standard error was reported from individual ANOVA analysis for each of Experiments 3-7.

4.3 Results

4.3.1 Emergence

4.3.1.1 Field

The emergence of 'Bolta' and 'Frontier' balansa clover seedlings in the field followed a generalised logistic curve from each sowing date (Appendix 3; Appendix 4). For example, for seeds sown on 3 July 2006, the maximum rate of emergence was on 20 July, 17 days after sowing. The rate then slowed until all seedlings had emerged (Figure 4.1).

The number of days from sowing until 50% of total emergence (DAS50) at different mean air temperatures during emergence formed two linear relationships. The first relationship between the DAS50 and mean air temperature showed a decrease in the number of days as temperature rose to an optimum. Beyond the optimum temperature, the number of DAS50 increased as temperatures increased further (Figure 4.2). For 'Bolta', the number of DAS50 decreased from 18.6 to 5.9 as mean 10 mm soil temperature between sowing and emergence increased from 4.1 to 8.5 °C and then increased to 20.5 as temperature increased to the maximum mean 10 mm soil temperature of 16.8 °C. The inverse of the DAS50 and temperature relationship was used to establish the rate of emergence in 'Bolta' and 'Frontier'. From this, T_{base} , and T_{max} were derived using the x-axis intercepts from the two linear relationships and T_{opt} from the intercept of the two lines. From calculations based on field data, both 'Bolta' and 'Frontier' had a T_{base} of ~2.2 °C, T_{opt} of 8.5 °C and T_{max} of 20.4 (± 4.6) °C for 'Bolta' and 18.8 (± 6.5) °Cd for 'Frontier' (Table 4.4). The thermal time to 50% emergence was then calculated using the slope of the line between T_{base} and T_{opt} to be 38 °Cd for 'Bolta' and 29 °Cd for 'Frontier'.

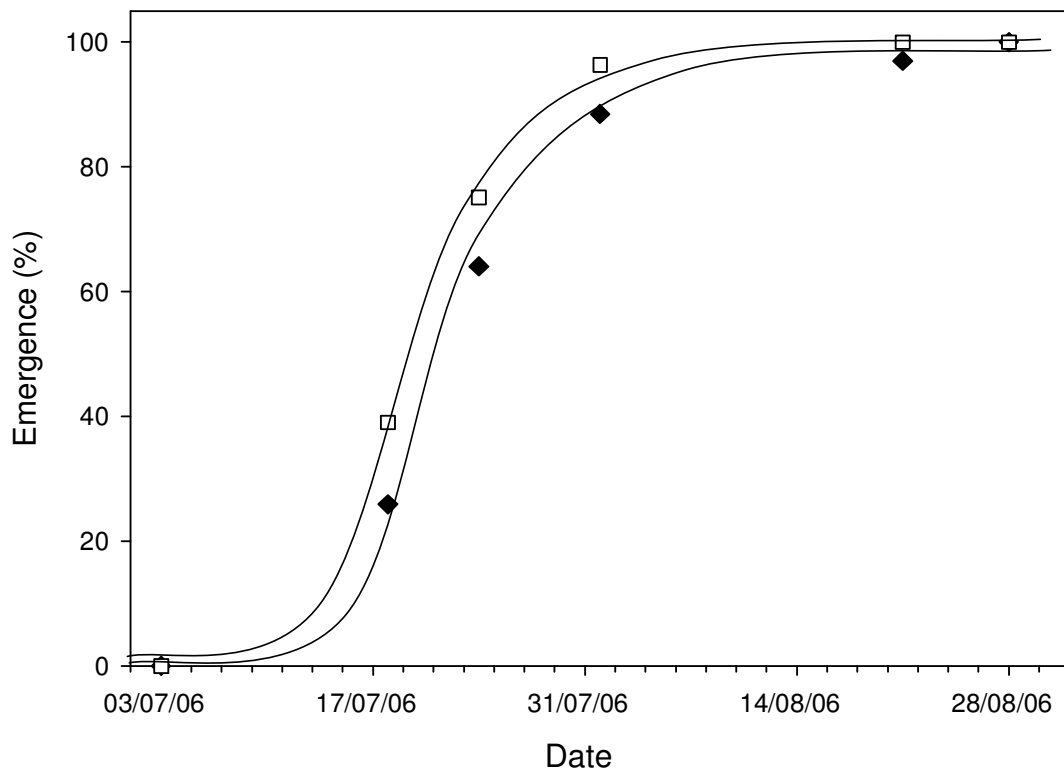


Figure 4.1 The emergence of 'Bolta' (◆) and 'Frontier' (□) balansa clover sown in the field on 3 July 2006 at Lincoln University, New Zealand.

Note: Lines are fitted generalised logistic functions from Equation 4.1. Coefficients are given in Appendix 4.

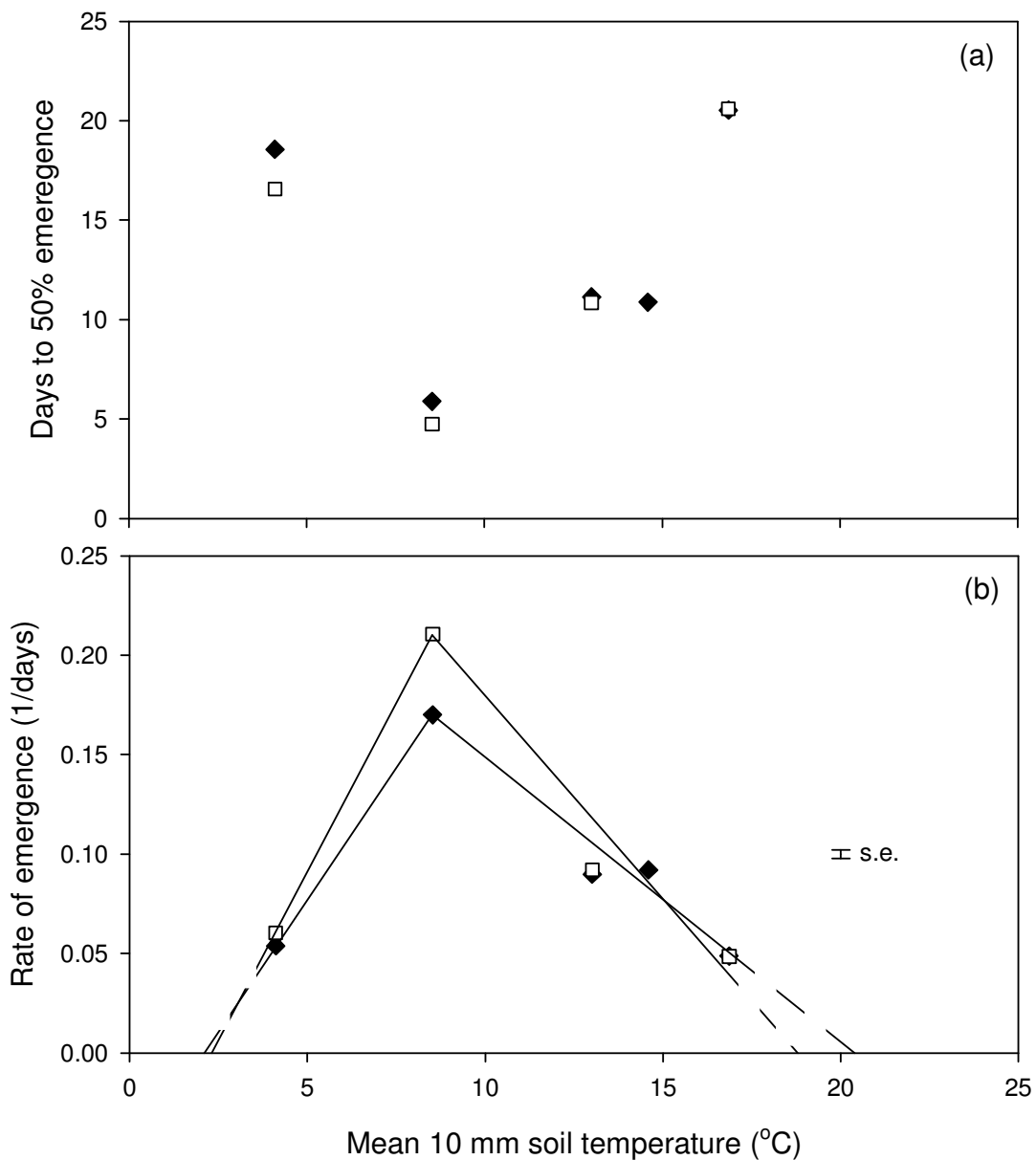


Figure 4.2 Number of days to 50% of final emergence (a) and emergence rate (b) of 'Bolta' (◆) and 'Frontier' (□) balansa clover against mean 10 mm soil temperature between sowing and emergence for seeds sown in the field on different dates (Table 4.2) at Lincoln University, New Zealand. Note: Lines are fitted linear regressions by least squares regression. Coefficients are given in Appendix 5. S.e. is the maximum standard error for the linear regressions.

Table 4.4 Base (T_{base}), optimum (T_{opt}) and maximum (T_{max}) temperature, thermal time (Tt) and thermal time with $T_{\text{base}} = 0\text{ }^{\circ}\text{C}$ for emergence of ‘Bolta’ and ‘Frontier’ balansa clover sown in the field at different times (Table 4.2) at Lincoln University, New Zealand.

	T_{base} ($^{\circ}\text{C}$)	T_{opt} ($^{\circ}\text{C}$)	T_{max} ($^{\circ}\text{C}$) (†)	Tt ($^{\circ}\text{Cd}$)	Tt ($T_{\text{base}} = 0\text{ }^{\circ}\text{C}$) ¹ ($^{\circ}\text{Cd}$)
‘Bolta’	2.1	8.5	20.4 (± 4.6)	38	59
‘Frontier’	2.3	8.5	18.8 (± 6.5)	29	56

Note: Coefficients are given in Appendix 5. † is 95% confidence interval, calculated when the data allowed. 10 mm soil temperatures were used. ¹Thermal time (Tt) analysis assumes a base temperature of $0\text{ }^{\circ}\text{C}$

4.3.2 First leaf appearance (Experiment 3-7)

The timing of the first leaf appearance was based on the linear relationship between the number of mainstem leaves against the number of days after sowing. The number of leaves on the mainstem increased linearly with days after sowing for ‘Aries’ perennial ryegrass, ‘Frontier’ balansa clover and ‘Laser’ Persian clover (Figure 4.4 and Appendix 7 and Appendix 8) in Experiments 3-7. The number of days from sowing until the appearance of the first leaf decreased for all species as the mean 10 mm soil temperature increased (Figure 4.3a). For example, ‘Frontier’ took 12 days for the first leaf to appear when mean 10 mm soil temperature was $11.5\text{ }^{\circ}\text{C}$, but decreased to 4 days as temperature rose to $23.8\text{ }^{\circ}\text{C}$. The subsequent rate of first leaf appearance increased as temperatures rose from the base temperature for each species (Figure 4.3b). Linear regression of the rate of first leaf appearance against mean 10 mm soil temperature allowed T_{base} and thermal time from sowing to first leaf to be calculated (Table 4.5). ‘Frontier’ balansa clover had a T_{base} for first leaf appearance of $2.4\text{ }^{\circ}\text{C}$ and a thermal time requirement of $97\text{ }^{\circ}\text{Cd}$.

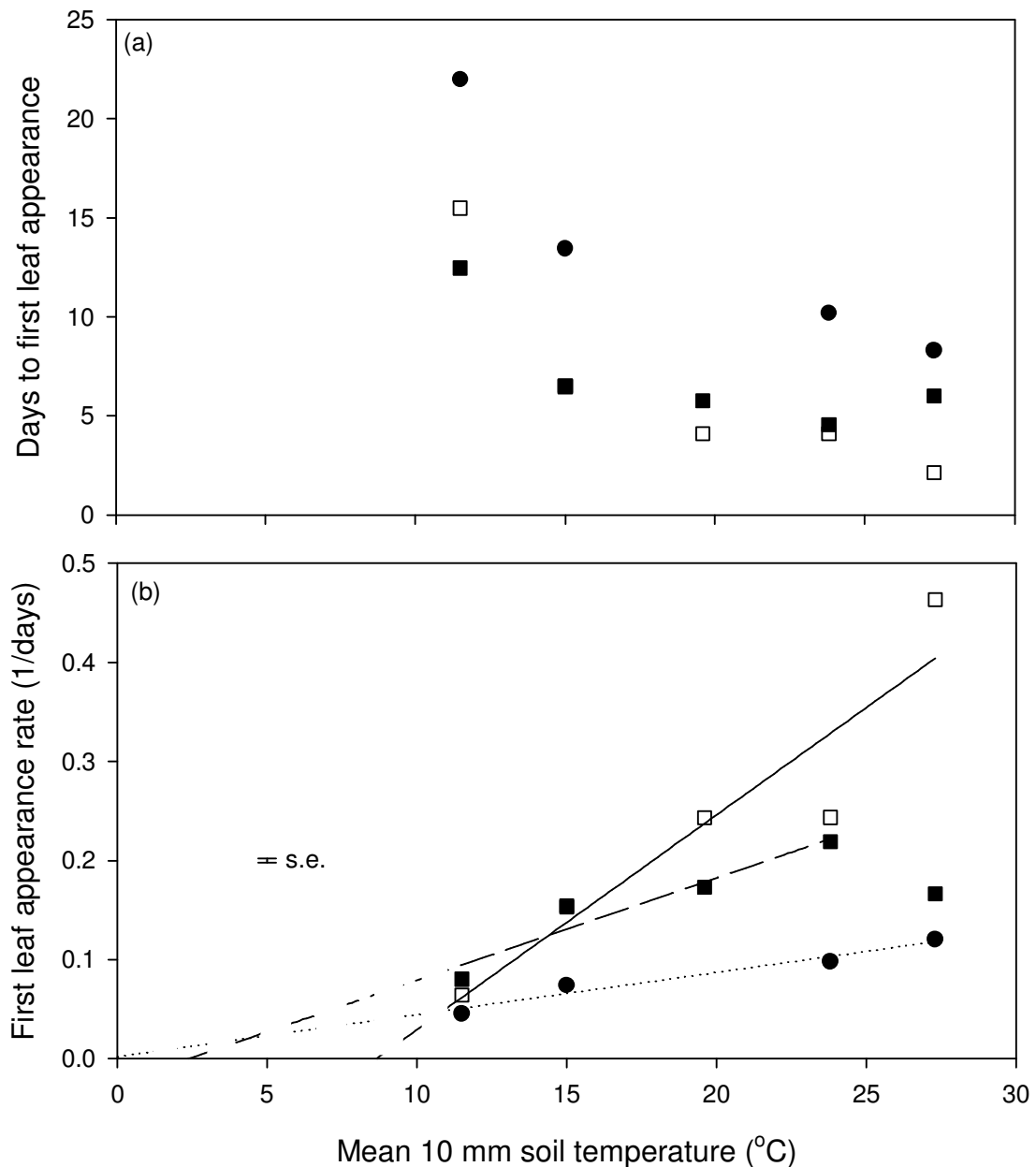


Figure 4.3 Number of days to the first leaf appearance (a) and the first leaf appearance rate (b) for 'Aries' (●, ⋯) perennial ryegrass, 'Frontier' balansa clover (■, - -) and 'Laser' Persian clover (□, —) grown in growth rooms at Lincoln University, Canterbury, New Zealand. Lines are linear regression by least squares.

Note: s.e. is the maximum standard error of the mean. Coefficients are given in Appendix 6.

Table 4.5 Base temperature (T_{base}) and thermal time requirements for first leaf appearance for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover grown in growth rooms at Lincoln University, Canterbury, New Zealand.

	T_{base} (°C) (†)	Tt (°Cd)	R^2 (%)	Tt ($T_{\text{base}}=0$ °C) ¹ (°Cd)	R^2 (%)
'Aries'	-0.5 (±9.6)	235	96	230	96
'Frontier'	2.4 (±9.4)	97	93	111	91
'Laser'	8.7 (±6.6)	46	87	95	71

Note: † is the 95% confidence interval. Coefficients are given in Appendix 6. ¹Thermal time (Tt) analysis assumes a base temperature of 0 °C

4.3.3 Phyllochron

4.3.3.1 Growth room (Experiment 3-7)

The number of leaves on the main stem increased linearly with days after sowing for each species at each temperature (Figure 4.4 and Appendix 7 and Appendix 8). The slope of each linear relationship was used to calculate the phyllochron (number of days/leaf) at each temperature (Figure 4.5). For each species, the rate of leaf appearance increased as temperature increased. For example, the rate of leaf appearance for 'Laser' Persian clover increased from 0.18 leaves/day to 0.48 leaves/day as the 10 mm soil temperature within the growth rooms increased from 11.5 °C to 23.8 °C (Figure 4.5b). The T_{base} calculated was between 3.1 (±4.3) °C for 'Aries' and 7.0 (±1.8) °C for 'Frontier' (Table 4.6). When measured in thermal time, the phyllochron for plants grown in growth rooms was estimated to be 51 °Cd for 'Aries', 38 °Cd for 'Frontier' and 42 °Cd for 'Laser'.

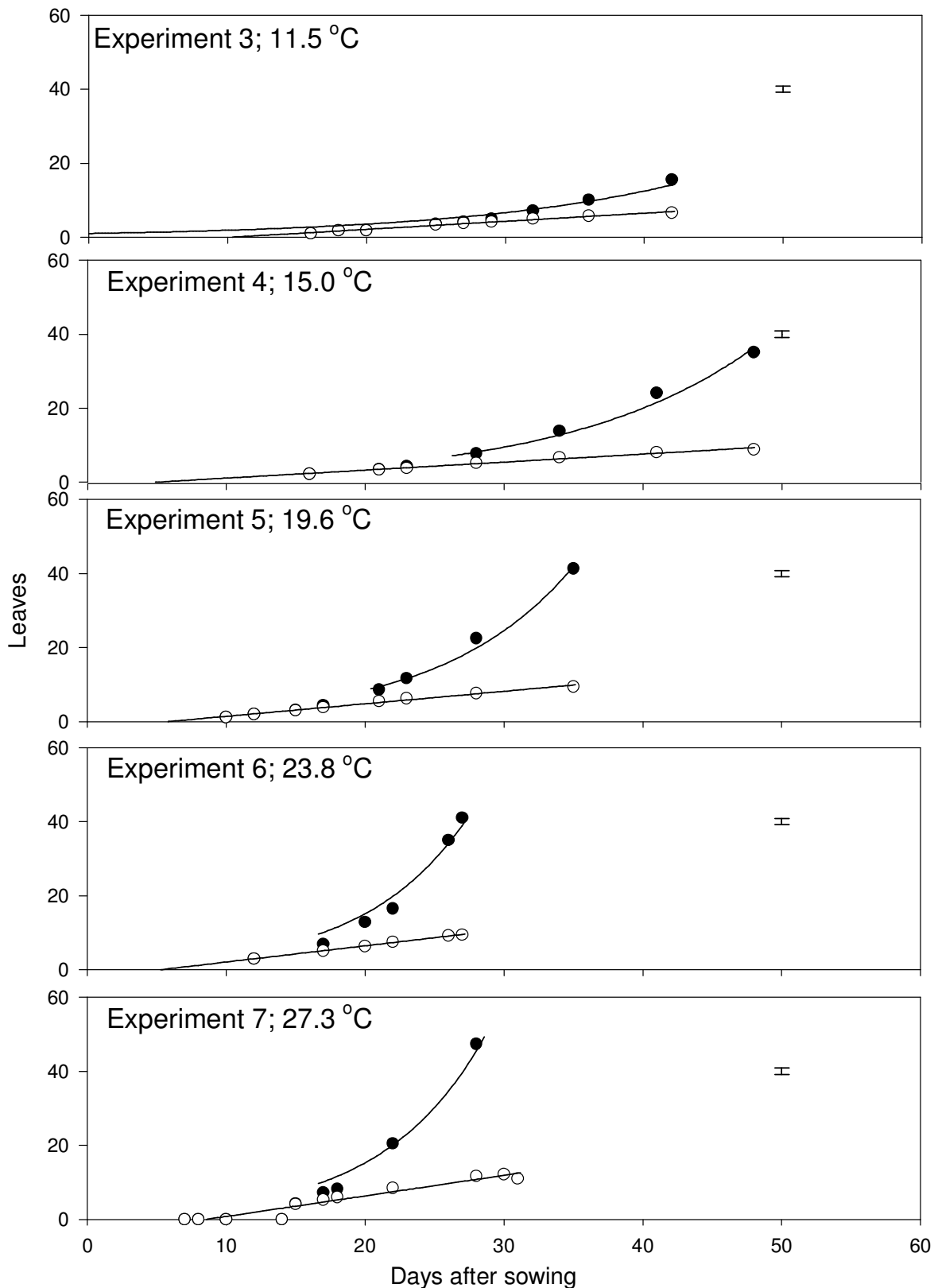


Figure 4.4 The number of mainstem (○) and total (●) leaves for 'Frontier' balansa clover sown in growth rooms.

Note: The linear relationships between days and mainstem leaves were fitted using least squares regression. The relationships between days and total leaves were fitted as exponential curves. Error bars are the standard error of means for final total leaf number. Coefficients are presented in Appendix 9 and Appendix 10.

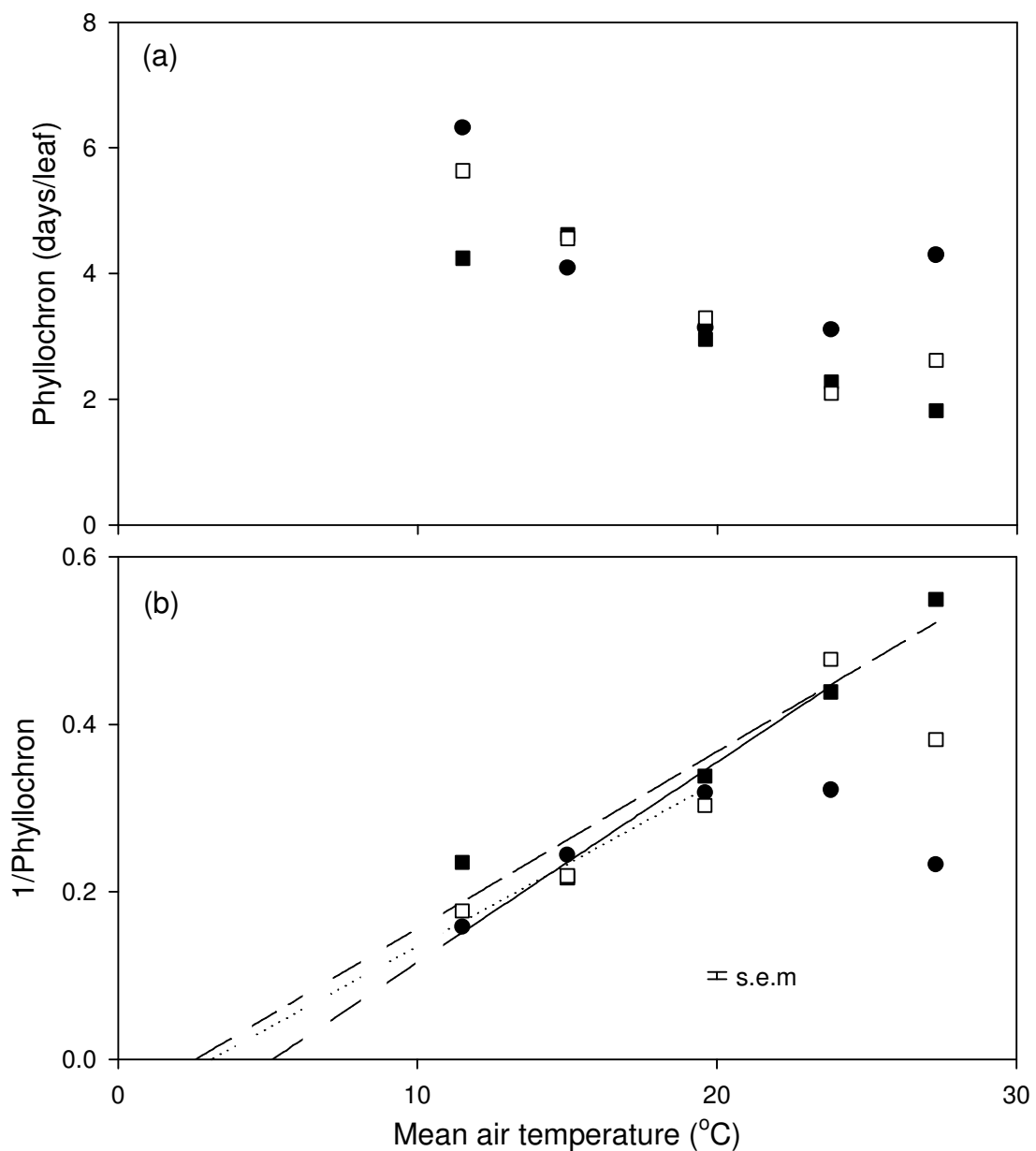


Figure 4.5 The mainstem phyllochron (a) and the leaf appearance rate (1/phyllochron) (b) for 'Aries' (●, ···) perennial ryegrass, 'Frontier' balansa clover (■, - -) and 'Laser' Persian clover (□, —) grown in growth rooms. Note: Lines are linear relationships by least squares regression. s.e.m. is the maximum standard error of the mean for the fitted lines. Coefficients for the fitted lines are presented in Appendix 11.

Table 4.6 Base temperature (T_{base}) and phyllochron of successive primary stem leaves for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover in growth rooms based on air temperatures.

	T_{base} (°C) (†)	Phyllochron (°Cd/leaf)	R^2 (%)	Phyllochron ($T_{\text{base}}=0$ °C) ¹ (°Cd/leaf)	R^2 (%)
'Aries'	3.1 (±4.3)	51	99	63	95
'Frontier'	7.0 (±1.8)	38	99	54 ²	90
'Laser'	5.2 (±8.2)	42	93	58	85

¹Thermal time (Tt) analysis assumes a base temperature of 0 °C, ² is not justified by the 95% confidence interval. † is the 95% confidence interval. Based on mean 1200 mm air temperatures.

4.3.3.2 Field (Experiment 2)

The appearance of leaves on the main stems of field-sown 'Bolta' and 'Frontier' balansa clover increased with days after sowing (Figure 4.6). The slope of each linear relationship was used to calculate the phyllochron (number of days/leaf) for each sowing date (Figure 4.7). The relationship between leaves/day (between T_{base} and T_{opt}) and temperature was strongest against air temperature, with a coefficient of determination (R^2) of 98% compared with an R^2 of 88% when 10 mm soil temperature was used.

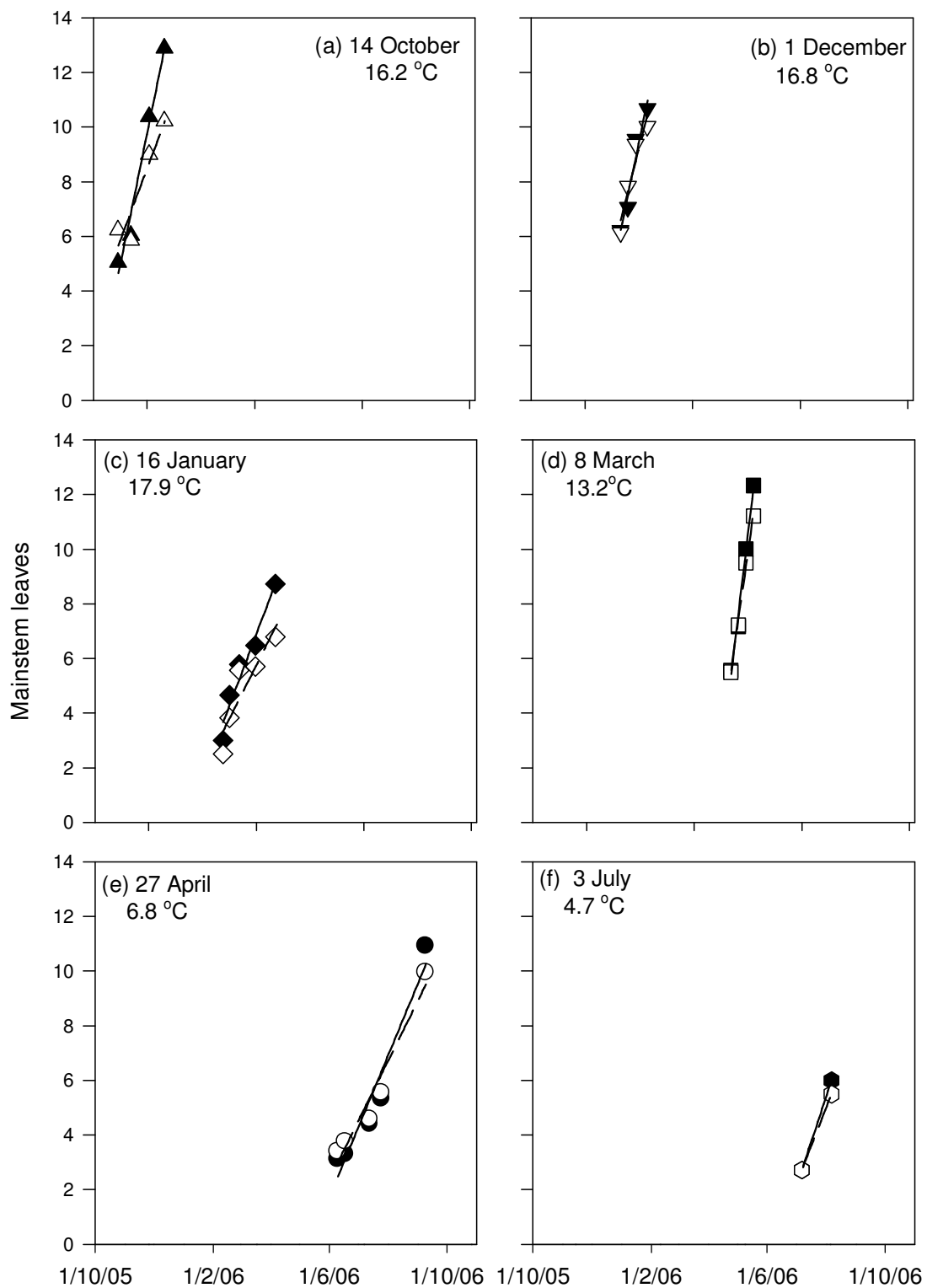


Figure 4.6 The number of leaves present on the mainstem of 'Bolta' (closed symbols) and 'Frontier' (open symbols) balansa clover sown after sowing on 14 October (\blacktriangle , \triangle), 1 December (\blacktriangledown , \triangledown), 16 January (\blacklozenge , \lozenge), 8 March (\blacksquare , \square), 27 April (\bullet , \circ) and 3 July (\bullet , \circ).

Note: Temperature shown is the mean air temperature between emergence and first trifoliolate leaf. Coefficients of determination for linear regressions (R^2) are $\geq 93\%$. Coefficients are presented in Appendix 12. For the year of sowing, see Table 4.2.

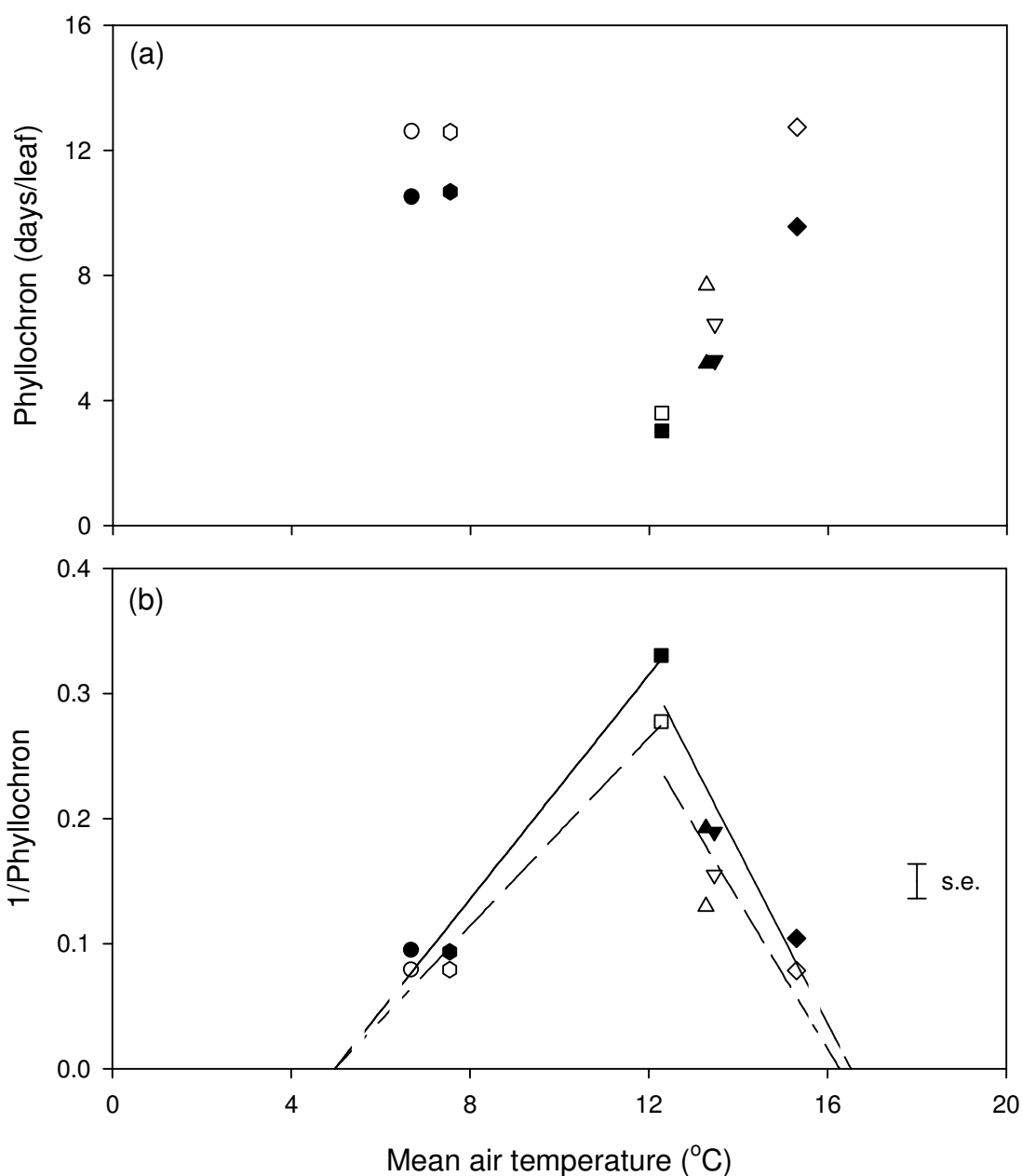


Figure 4.7 The (a) phyllochron of 'Bolta' (▲,▼,◆,■,●,○) and 'Frontier' (△,▽,◇,□,○,e) balansa clover sown at different times in the field against the mean air temperature between emergence and first trifoliolate leaf appearance emergence and (b) the inverse relationship. Plants were sown on 14 October (▲,△), 1 December (▼,▽), 16 January (◆,◇), 8 March (■,□), 27 April (●,○) and 3 July (●,○).

Note: Lines are linear regression by least squares regression for 'Bolta' (—) and 'Frontier' (---). Coefficients are presented in Appendix 13. s.e. is the maximum standard error for the fitted lines. For the year of sowing, see Table 4.2.

From these relationships, the cardinal temperatures and thermal time per leaf were calculated (Table 4.7). For ‘Frontier’ balansa clover, the T_{base} was estimated to be 5.0 °C, T_{opt} ~12 °C and T_{max} 16.5 °C. When $T_{base} = 0$ °C, the phyllochron was calculated to be 47 °Cd, or 7 °Cd less than that reported from the growth room (Table 4.6).

Table 4.7 The phyllochron and cardinal temperatures for ‘Bolta’ and ‘Frontier’ balansa clover sown in the field at different times at Lincoln University, Canterbury, New Zealand.

	T_{base} (°C) (†)	Phyllochron (°Cd/leaf)	R^2 (%)	T_{opt} (°C)	T_{max} (°C) (†)	R^2 (%)	Phyllochron ($T_{base}=0$ °C) ¹ (°Cd/leaf)	R^2 (%)
‘Bolta’	5.0 (±2.9)	22	98	~12	16.5 (±2.4)	88	47 ²	69
‘Frontier’	5.0 (±2.8)	27	98	~12	16.2 (±3.1)	69	56 ²	69

Note: ¹Thermal time (Tt) analysis assumes a base temperature of 0 °C. † is the 95% confidence interval. Coefficients are given in Appendix 13. ²is not justified by the 95% confidence interval.

4.3.4 Appearance of axillary leaves

4.3.4.1 Field (Experiment 2)

The number of days to axillary leaf appearance was recorded in ‘Bolta’ and ‘Frontier’ balansa clover in the field from multiple sowing dates. The number of days from emergence until axillary leaves appeared decreased as the mean air temperature between sowing and flowering increased in both cultivars (Figure 4.8a). For example, ‘Bolta’ took 80 days for the appearance of axillary leaves at 5.9 °C but this decreased to 14 days at 15.7 °C.

The rate of axillary leaf initiation increased exponentially (Figure 4.8b) for ‘Bolta’ ($R^2 = 99\%$) and for ‘Frontier’ ($R^2 = 99\%$) as the mean air temperature increased. The T_{base} and Tt were then estimated from a linear relationship fitted to the approximately linear portion of the curve, corresponding to mean air temperatures ≥ 12 °C. Using the linear analysis method, the T_{base} for axillary leaf appearance was estimated to be 9.1 °C (±4.5) for ‘Bolta’ ($R^2 = 83\%$) and 9.0 (±2.8) °C for ‘Frontier’ balansa clover ($R^2 = 94\%$) (Table 4.8).

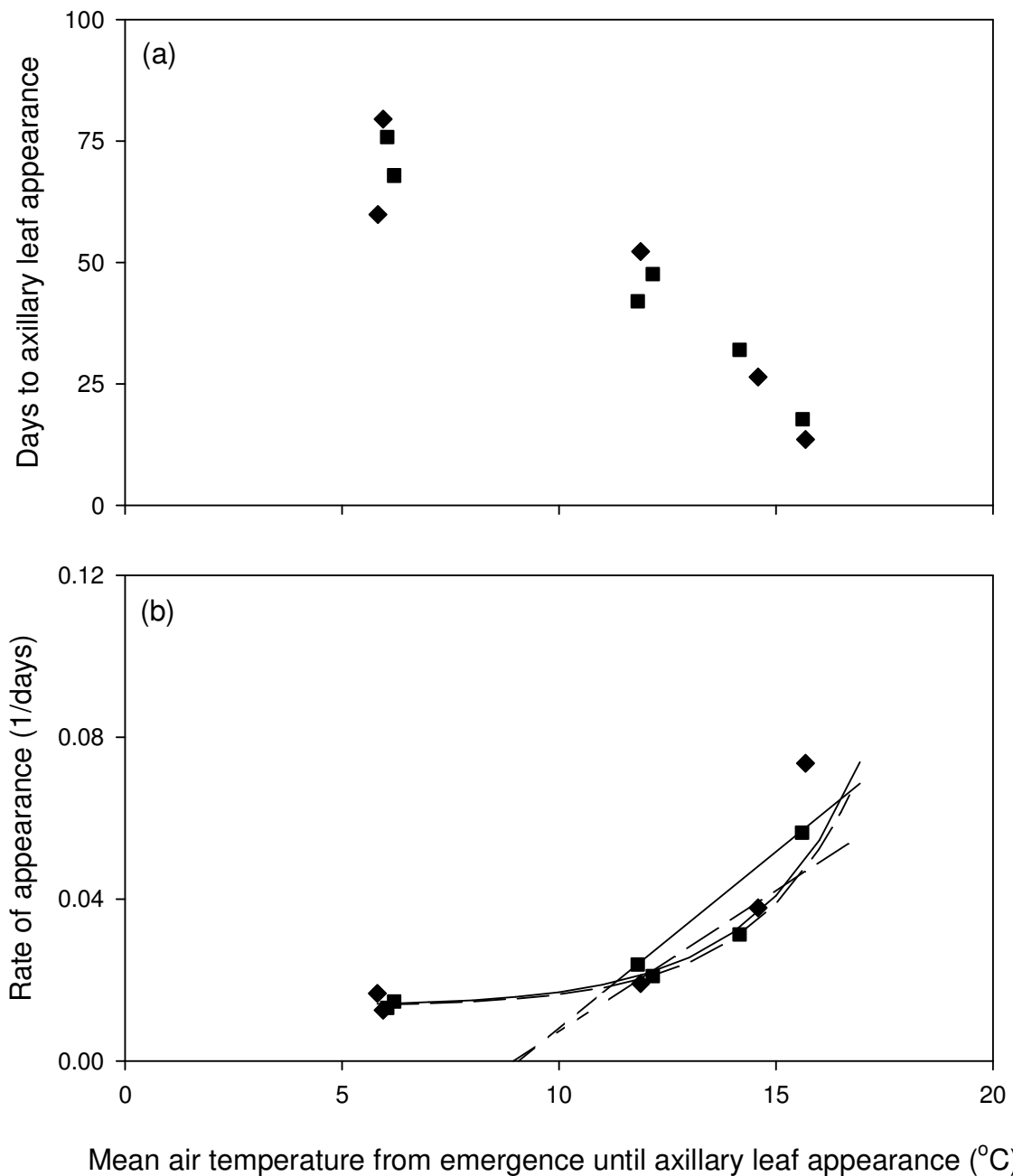


Figure 4.8 Number of days to the appearance of axillary leaves (a) and rate of axillary leaf appearance against mean air temperature from emergence to the appearance of axillary leaves (b) of 'Bolta' (◆) and 'Frontier' (■) balansa clover sown at different times at Lincoln University, Canterbury, New Zealand.

Note: Lines are exponential curves (Equation 4.4) for the total data range, and linear regressions for temperatures ~ 12 °C for 'Bolta' (—) and 'Frontier' (---). Coefficients are presented in Appendix 14.

Table 4.8 The base temperature (T_{base}) and thermal time (Tt) requirements for axillary leaf appearance in 'Bolta' and 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand.

	T_{base} (°C) (†)	Tt (°Cd)	R^2 (%)	Tt (°Cd) ($T_{base} = 0$ °C)	R^2 (%)
'Bolta'	9.1 (± 4.5)	115	83	318 ¹	48
'Frontier'	9.0 (± 2.8)	144	94	399 ¹	55

Note: Thermal time was calculated using mean 1200 mm air temperature. † is 95% confidence interval. ¹Not justified by 95% confidence interval.

4.3.4.2 Growth room (Experiment 3-7)

The number of days from emergence until axillary leaves appeared differed for each species as the temperature in the growth room changed (Figure 4.4, Appendix 7 and Appendix 8). The number of days from emergence until the appearance of axillary leaves decreased in all species as the mean air temperature increased from 6.8 °C to 15.6 °C (Figure 4.9a). For example, 'Frontier' took 19 days for the appearance of axillary leaves at 6.8 °C but this decreased to 11.8 days at 15.6 °C.

The rate of axillary leaf initiation increased for 'Aries' ($R^2 = 99\%$) 'Frontier' ($R^2 = 100\%$) and 'Laser' ($R^2 = 94\%$) as the mean air temperature increased (Figure 4.9b). The T_{base} and Tt were then estimated from a linear relationship fitted to the increasing portion of the curve. Using this method, the T_{base} for axillary leaf appearance was estimated (Table 4.9). The thermal time for the appearance of axillary leaves, was estimated to be 282 °Cd for 'Aries', 230 °Cd for 'Frontier' and 274 °Cd for 'Laser' when base temperature was 0 °C.

Table 4.9 Base temperature (T_{base}) and thermal time (Tt) from sowing for axillary leaf appearance for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover grown in growth rooms.

	T_{base} (°C) (†)	Tt (°Cd)	R^2 (%)	Tt ($T_{base}=0$ °C) (°Cd)	R^2 (%)
'Aries'	5.3 (± 5.6)	190	99	282	99
'Frontier'	-1.1 (± 1.4)	244	100	230	100
'Laser'	2.2 (± 14.1)	236	94	274	99

Note: † is 95% confidence interval. Coefficients are presented in Appendix 15.

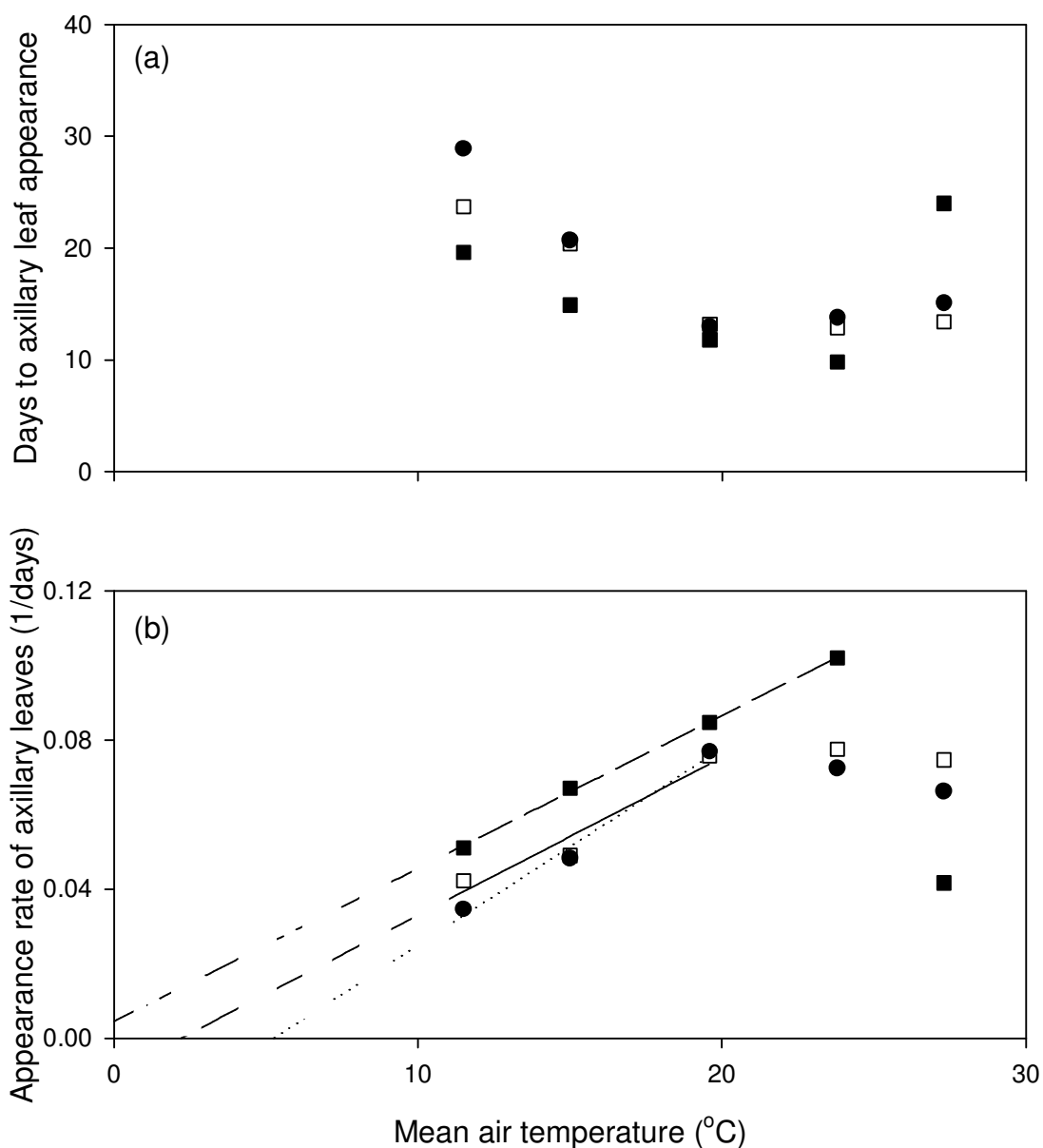


Figure 4.9 The number of days to the appearance of axillary leaves (a) and rate of axillary leaf appearance (b) for 'Aries' (●, ...) perennial ryegrass, 'Frontier' balansa clover (■, - -) and 'Laser' Persian clover (□, —) grown in growth rooms at Lincoln University, Canterbury, New Zealand.

Note: Lines are linear regression by least squares. Coefficients are presented in Appendix 15.

4.3.5 Leaf area and seedling weight

4.3.5.1 Growth room (Experiment 3-7)

The relationship between leaf area and above ground dry weight increased linearly for all species up to 720 °Cd (minimum $R^2 = 94\%$) (Figure 4.10). Balansa and Persian clover increased leaf area at a rate of 197 cm^2/g dry matter ($R^2 = 94\%$) (95% CI at 1.0 g dry weight). 'Aries' perennial ryegrass produced leaf area at 96 cm^2/g dry matter.

The specific leaf weight (g/cm^2) of the ryegrass peaked at 0.01 g/cm^2 and was higher ($P < 0.001$) than balansa or Persian clovers throughout the period (Figure 4.11a). The green area per leaf for Persian clover peaked at 8.8 cm^2/leaf at 720 °Cd after emergence and was higher ($P < 0.001$) than for balansa or ryegrass throughout the period (Figure 4.11b). This was observed in Persian clover as larger, thinner leaves compared with balansa clover or ryegrass.

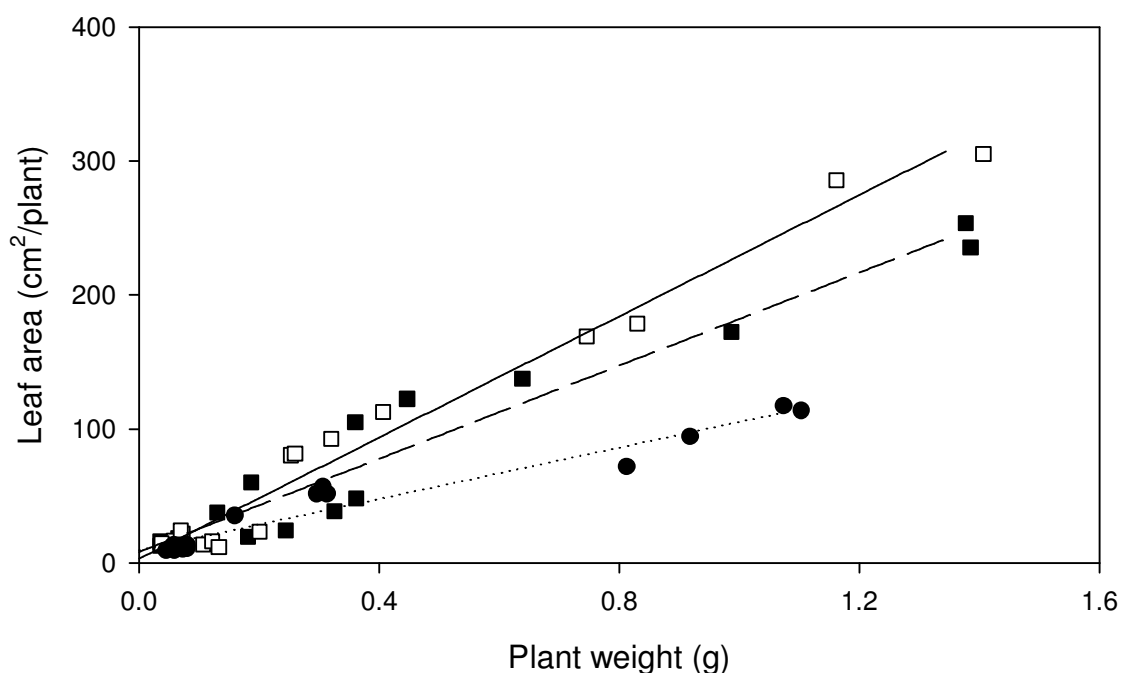


Figure 4.10 Total above ground green leaf area at different dry weights for 'Aries' (●, ⋯) perennial ryegrass, 'Frontier' balansa clover (■, - -) and 'Laser' Persian clover (□, —) grown in growth rooms at Lincoln University, New Zealand.

Note: Lines are linear regression by least squares ($R^2 \geq 94\%$). Coefficients are presented in Appendix 16.

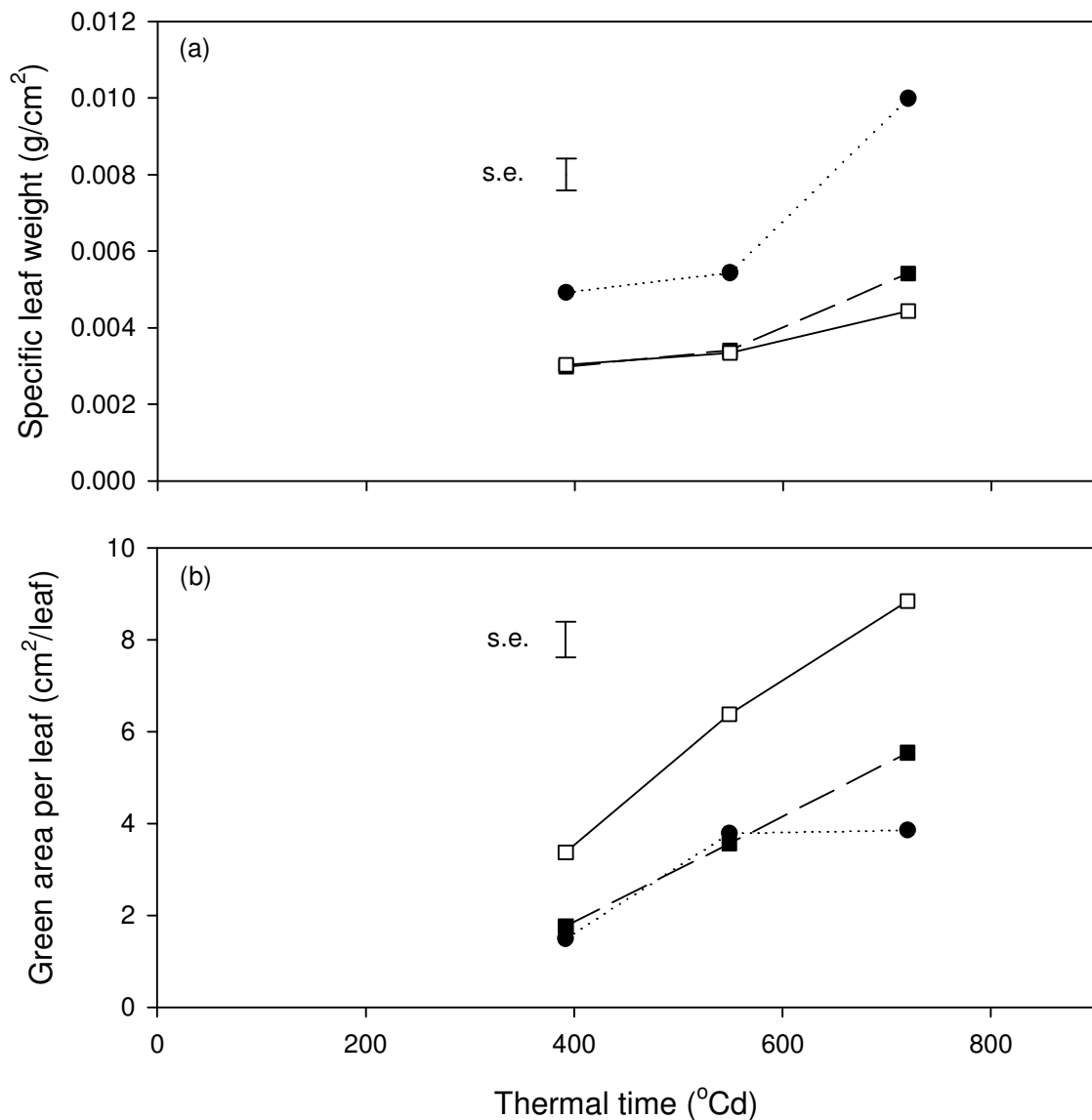


Figure 4.11 The (a) specific leaf weight (g/cm^2) and (b) green area per leaf (cm^2/leaf) for 'Aries' (●, ...) perennial ryegrass, 'Frontier' balansa clover (■, - -) and 'Laser' Persian clover (□, —) against thermal time from emergence ($T_{\text{base}} = 0\text{ }^\circ\text{C}$) grown in growth rooms at Lincoln University, New Zealand. Note: s.e. is the maximum standard error.

4.4 Discussion

The most appropriate way to describe the timing of different vegetative phenophases of balansa clover was to use thermal time. The temperature the plant perceived during each stage of development was able to account for the differences in the timing of phenophases observed when plants were sown in different climatic conditions. The six experiments, in the field and in the growth room, complemented

one another by providing contrasting conditions which were targeted at quantifying the timing and duration of different phenophases.

4.4.1 Field Emergence

The field sown balansa clover in Experiment 2 was used to validate the timing of emergence. By having cooler temperatures in the field, data from Experiment 2 were used to validate the estimate of T_{base} made in the growth room, where with the lowest mean soil temperature was 11.5 °C despite being set to 6 °C (Appendix 2a). The mean 10 mm soil temperatures recorded in the field between sowing and emergence were as low as 6.4 °C for plants sown on 3 July (Figure 4.2). The T_{base} for emergence of balansa clover was calculated to be ~2.2 °C (Table 4.4). This means that balansa clover which is sown or is naturally regenerating when soil temperatures are below 2.2 °C would not accrue thermal units towards emergence. Balansa clover sown in cool soil temperatures on 3 July at Lincoln University (Experiment 2) took 19 days to reach 50% of total emergence. That period represents 19 days which allows other species, with a lower T_{base} , to emerge and to begin capturing resources at the expense of balansa clover. The estimate of base temperature was comparable to other temperate legumes which have been reported in the literature (Moot *et al.*, 2000; Lonati *et al.*, 2009). However, because there was only one temperature in the field below T_{opt} , the estimate of T_{base} was made using only 2 data points for each balansa clover cultivar (Figure 4.2). More experimental data are required to give an accurate, statistically sound estimate of T_{base} . In coastal Canterbury, this would mean more sowing dates between May and August.

For rapid emergence of balansa clover, 9.7 °C soil temperatures are optimum (Table 4.4). However, emergence is only one phase of seedling establishment. Plants sown on 27 April experienced mean 10 mm soil temperatures of 10.2 °C and emerged in the quickest time of 6 days. However, while plants sown on 8 March took twice as long to emerge (11 days), they had 7 leaves on the mainstem before the 27 April sown plants had even emerged. The optimum sowing strategy, therefore, is not solely dependent on the rate at which a species emerges (Section 2.3).

Unexpectedly, 'Frontier' showed no response to temperature in the growth room and took ~6 days to emerge regardless of the mean temperatures. The most likely explanation is there was a problem with the methods used (Section 4.2.2.1) (Jansen and Ison, 1994b). The seed probably imbibed water and began the germination process before Experiments 4 to 7 were sown in the growth room. 'Frontier' balansa clover seed was washed to remove lime coating applied by the supplier immediately prior to Experiment 4, 15 °C. Seeds were wetted and vigorously rubbed against steel mesh to remove the coating and may have imbibed water at that time. The time between the seed being washed and sown varied between the growth room experiments and seeds were stored between sowings at temperatures >15 °C. This would have effectively decreased the number of thermal units from sowing to emergence recorded in the growth room, down from the ~60 °Cd recorded in the field. Seed for the field experiment was not coated and therefore washing did not occur.

4.4.2 Growth room temperatures

The temperatures recorded within the growth room during Experiments 3-7 varied between the air, 10 mm soil and soil surface temperature measurements sites (Appendix 2). The 10 mm soil temperature and soil surface temperature increased above air temperature as the room's electronically controlled sequential lighting system came on and produced a radiant heat load. The temperatures then decreased as the lights were turned off. For example, in Experiment 3 the mean air temperature was 6.8 °C and ranged 1.5 °C, from 5.8 to 7.3 °C. In the same experiment, mean 10 mm soil temperature was 11.5 °C and ranged 10.0 °C, from 6.2 to 16.2 °C. The mean thermal time accumulated each day for air and 10 mm soil temperature for Experiments 3-7 is given in Table 4.3.

All results from growth room experiment-based data for soil temperature dependent seedling development calculations may have been influenced by the temperature schedule used. The Conviron PGV36 growth rooms used for Experiments 3-7 were unable to produce mean daily 10 mm soil temperatures below 11.5 °C. The radiant heat from the lighting system lifted the soil temperature above the average air temperature of 6.8 °C. Because of this, all base temperatures from emergence to the

appearance of axillary leaves below 11.5 °C in the growth rooms came from extrapolated estimates beyond the temperature range available.

4.4.3 Phyllochron

Leaves appeared on the mainstem at a rate constant in thermal time for both field and growth room experiments. The consistent appearance of leaves after a given number of thermal units confirmed phyllochron as a cultivar specific process. There was minor variation between the balansa cultivars (Figure 4.7b), and suggests the expression of these genetic traits may be useful as selection criteria for future development when drawing from wider genetic material. For example, cultivars could be selected for a shorter phyllochron which would increase the number of mainstem leaves for any given time period.

The base temperature for mainstem leaf appearance (phyllochron) was estimated to be 5.0 °Cd for both 'Bolta' and 'Frontier', but with limited data below T_{opt} , the validity of this estimate is questionable. The data available for subterranean clover and implies that the T_{base} for leaf development is equal to or lower than that for germination and emergence (Moot *et al.*, 2000; Moot *et al.*, 2003a). This is also true for white clover and Caucasian clover (Black *et al.*, 2006a). It is therefore proposed future work with the phyllochron of balansa clover should assume a T_{base} of 2.5 °C, the same as the other development phases, until more comprehensive data can be generated.

4.4.4 Leaf area and seedling weight

'Frontier' balansa clover and 'Laser' Persian clover seedlings produced leaf area at a greater rate per gram of above ground dry matter than 'Aries' perennial ryegrass seedlings (Figure 4.10). The ability of 'Frontier' to emerge and capture light in a pasture is therefore dependent on the ability to produce a large number of small leaves quickly. This contrasted with 'Laser', which produced fewer, larger leaves per unit weight (Figure 4.11b). Black (1958) showed large leaved subterranean clover cultivars produced fewer leaves compared with small leaved cultivars, while maintaining similar total leaf area. They suggested that large leafed cultivars were

anecdotally considered higher yielding, and that the difference in productivity was due to heavier embryos driving cotyledon area and dry weight (Chapter 7). Balansa clover grazing management suggestions from Australia are targeted towards strong grazing pressure from emergence (Kelly and Mason, 1986; Dear *et al.*, 2002). The ability for balansa clover to produce leaves more quickly than the species it is competing with, e.g. perennial ryegrass or subterranean clover, is likely to confer an advantage over other species in a set-stock environment. Each leaf which is grazed has effectively 'cost' the balansa clover plant less in terms of leaf area than the heavier leaved Persian clover or subterranean clover.

Future work could be based around relating leaf size to mainstem node position and leaf area index (LAI). The rate of leaf appearance may diminish as the plant ages and diverts resources from initiating leaf primordia into expanding existing leaves (Brown *et al.*, 2005) or as available light limits seedling growth. This may be what is seen in 'Laser' Persian clover (Figure 4.12c) where the total number of leaves remain linear ($R^2 = 92\%$), rather than the expected exponential increase in appearance rate ($R^2 = 89\%$, Figure 4.12c). This may coincide with internode elongation and reallocation of photosynthetic assimilates to the root structures or reproductive development. Clerget *et al.* (2008) and Brown (2005) identified the change in assimilate partitioning as the likely driver of the change in phyllochron observed in sorghum (*Sorghum japonicum*) and lucerne.

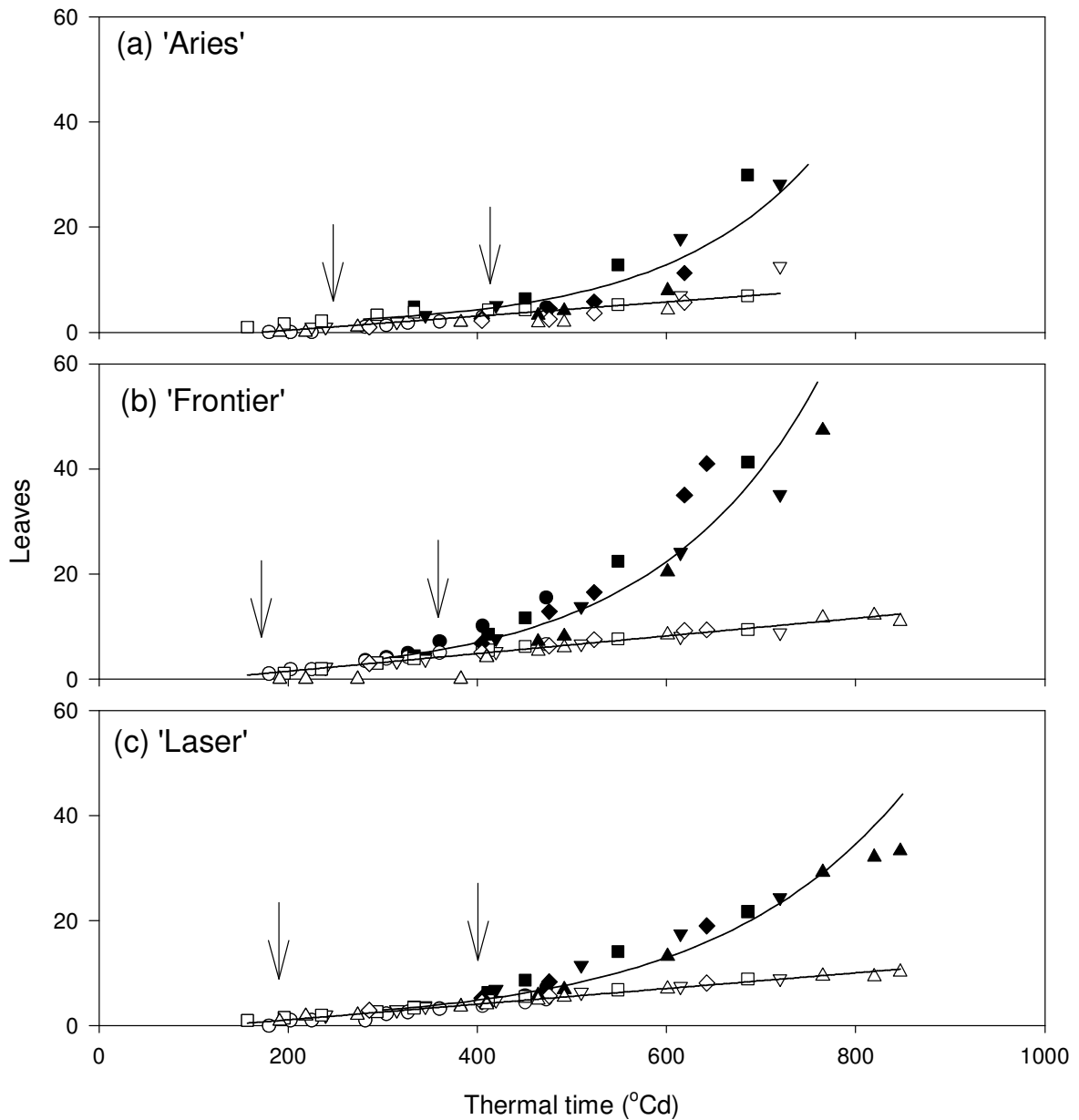


Figure 4.12 The number of mainstem (○□△▽◇) and total (●■▲▼◆) leaves for (a) 'Aries' perennial ryegrass, (b) 'Frontier' balansa clover and (c) 'Laser' Persian clover against accumulated thermal time from sowing of five growth room experiments set at different temperatures. Exp. 3 (●,○), Exp. 4 (▼,▽), Exp. 5 (■,□), Exp. 6 (◆,◇), and Exp. 7 (▲,△). Arrows indicate the time of first leaf appearance and axillary leaf appearance. Note: Thermal time sums are calculated using 1200 mm air temperature with $T_{base} = 0\text{ }^{\circ}\text{C}$.

4.4.5 A comparison of temperature measurement location

In the field, the timing of emergence of 'Bolta' and 'Frontier' balansa clover was more accurately predicted using the mean air temperature between sowing and emergence than with mean 10 mm soil temperature. The comparison of the two temperatures was based on the rate of emergence and temperature between T_{opt} and T_{max} (Appendix 5), because of the limited data for emergence in the field below T_{opt} (2 points). Mean 10 mm soil temperature was chosen for the analysis because of its proximity to the site of temperature perception (Peacock, 1975). Using mean air temperature (Stevenson screen) gave similar but lower estimates of thermal time requirements for emergence of 18.3 °Cd for 'Bolta' and 17.6 °Cd for 'Frontier' compared with those values given in Table 4.4, because of the lower temperatures recorded at Stevenson screen height, but could be used as a satisfactory substitute. This decrease in calculated thermal time requirement when using air temperature gave the same result as reported by Moot *et al.* (2000). These data showed mean air temperature (Stevenson screen) from sowing to emergence was an adequate estimate of the temperature perceived by the plant to influence the rate of emergence with $R^2 \geq 95\%$ for mean air temperature compared with $R^2 \geq 94\%$ for mean 10 mm soil temperature. This may not be the case in an environment where soil temperature and air temperature are more divergent.

The duration of each phenophase beyond emergence was calculated using air temperature, even for the formation of the first leaf when plants were near to the soil surface. Throughout the recording of the vegetative development in the growth rooms (Experiments 3-7), plants never exceeded 200 mm in height (estimated) and were constantly thinned to avoid intra-plant competition. In the field (Experiment 2), the plants rarely exceeded 300 mm (estimated) during the measurement period. This may explain why the single temperature measurement location (1200 mm air temperature) was able to adequately describe the timing of the phenophases studied. Had leaf appearance been recorded beyond the time of canopy closure, contrasting microclimates would have formed above, within and below the canopy which would have led to the need to move the temperature measurement site (Tanner, 1963; Jamieson *et al.*, 1995b). Daily air temperature has been previously used for satisfactory analysis of phenological development in temperate pasture species (Moot *et al.*, 2000; Lonati *et al.*, 2009).

4.4.6 Calculation of base temperature

Using the 95% confidence interval test on T_{base} estimates to determine if they are truly different from 0 °C highlights the need for considered understanding of the calculation and application of base temperatures. Base temperature is calculated using an extrapolation of the linear relationship between rate and temperature for each phenophase (referred to throughout this thesis as T_{base}). The method requires the exclusion of the period of exponential increase in the rate of development at temperatures which approach the minimum threshold (Figure 4.8) (Angus *et al.*, 1981). Therefore, T_{base} does not represent the lowest temperature at which each particular development process will occur. Estimates of T_{base} discard the period when enzymatic activity is increasing exponentially as temperature rises above the true base temperature threshold by way of the Arrhenius equation and Q_{10} calculations (Smith *et al.*, 1999). This period appeared to occur at temperatures below those used in the experiments here, except for the appearance of axillary leaves in the field.

In the field (Experiment 2), the rate of axillary leaf appearance for 'Bolta' and 'Frontier' increased at an exponential rate through the temperature range for both species ($R^2 = 99\%$). A linear relationship between rate and temperature was formed at mean 1200 mm air temperatures of $\sim \geq 12$ °C (Figure 4.8). By using these temperatures, T_{base} was estimated at ~ 9 °C, however axillary leaves appeared on plants in ~ 6 °C temperatures in both 'Bolta' and 'Frontier'.

This also highlights the idea that T_{base} is only an approximation of the true temperature threshold (Angus *et al.*, 1981). In most situations, estimating T_{base} from the linear portion of the rate response curve is able to accurately capture the point when development does stop. However, some development can occur at or below the estimated T_{base} . To overcome this weakness in temperate climates, where temperatures are often between T_{base} and T_{opt} , the traditional method of accumulating thermal time of the average of maximum and minimum daily temperature minus the base temperature has been modified. Two basic methods have been developed. The first method, used here, involves modelling the daily temperature profile in eight slices and integrating them to give the daily thermal time accumulation. It describes

the true sigmoid shape of daily temperature fluctuations (e.g. Appendix 2), rather than assume half the day is spent at the maximum temperature and half is at the minimum (Jones and Kiniry, 1986). The other method is the development of a 'broken stick' response curve to describe the increased amount of thermal time accumulated at low temperatures. When modelling maize production in a cool climate like Lincoln, New Zealand, Wilson *et al.* (1995) proposed a two-phase linear regression to modify the amount of thermal time accumulated at temperatures below the inflection point ($\leq T_i$). They decreased the amount of thermal time accumulated for each unit of temperature between 0 °C and T_i to maintain a linear relationship between thermal time and development. The same method was used by Brown *et al.* (2005) for lucerne development.

One application of this method of calculating T_{base} from a linear regression is to generate an estimate of T_{base} using very few measurements. For example, Figure 4.2 was used to calculate the T_{base} for 50% emergence of 'Bolta' and 'Frontier' balansa clover based on two points. The temperatures at which the measurements were taken (4.1 and 8.5 °C) fell between estimates of T_{base} and T_{opt} for germination. However, because there were only two points, no standard error could be calculated and there were no assurances that the values did fall within T_{base} and T_{opt} for emergence. Some seeds which do germinate may subsequently fail to emerge because of predation by soil borne organisms or through lack of carbohydrate reserves, making them abnormal seedlings (International Seed Testing Association, 1999). Because of this, the two processes cannot be assumed to have the same cardinal temperatures although it is highly likely. It is important, therefore, when viewing T_{base} and thermal time estimates, to understand what the data are presenting. T_{base} is the temperature at which development would begin a linear increase in rate, were the linear portion of the rate curve extrapolated. The amount of thermal time required to progress between two sequential stages of development is calculated when temperatures are between T_{base} and T_{opt} . Comparing thermal time requirements between species or phenophases requires the base temperature to be accounted for and is done through the use of $T_{base} = 0$ °C re-analysis when appropriate (Moot *et al.*, 2000).

4.5 Conclusions

The experiments described in this chapter have been used to quantify the timing of a range of development stages in 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover. Specific conclusions are:

- Thermal time was the main driver of development rate across all species and in all stages reported here.
- 'Frontier' balansa clover had a:
 - T_{base} for emergence of 2.3 °Cd and, based on 10 mm soil temperatures, required 29 °Cd to reach 50% emergence. When $T_{\text{base}} = 0$ °C; 56 °Cd.
 - T_{base} for first leaf appearance of 2.4 (± 9.4) °C and, based on 1200 mm air temperatures, a thermal time requirement of 97 °Cd. When $T_{\text{base}} = 0$ °C; 111 °Cd.
 - T_{base} for successive mainstem leaf appearance of 7.0 (± 1.8) °C and a phyllochron of 38 °Cd. When $T_{\text{base}} = 0$ °C; 54 °Cd.
 - T_{base} for axillary leaf production in the field of -1.1 (± 8.1) °C and the first axillary leaf appeared after 537 °Cd. When $T_{\text{base}} = 0$ °C; 399 °Cd.
- From this, T_{base} was estimated to be ~ 2.5 °C for all processes. Any disagreements were ultimately proposed to be due to the lack of temperatures available below the optimum temperature.
- Few estimates of base temperature for each phenophase were different from 0 °C (95% CI). Using $T_{\text{base}} = 0$ °C would give satisfactory estimates of the thermal time requirements for each stage of vegetative development, except for first leaf appearance.
- Using 1200 mm air temperature gave a satisfactory relationship with each of the phenophases and could be used to establish cardinal temperatures for all vegetative development. The relationships may not hold as plants approach canopy closure.
- After 720 °Cd, 'Frontier' had 35 total leaves, each with a specific leaf weight of 0.0054 g/leaf with 5.54 cm²/leaf.

5 Reproductive development

5.1 Introduction

In Chapter 3, the success of balansa clover establishment in a mixed sward was shown to depend on management to maximise seed set in the first year of growth and to allow re-establishment in each new season. Chapter 4 followed this with the quantification of the physiological development for emergence, leaf appearance rate, the appearance of axillary leaves and early leaf area production. This chapter aims to define the duration of vegetative period by identifying the time of flowering and seed maturity. This information will help form grazing management recommendations for the spring and summer seasons.

The time of flowering in balansa clover was initially calculated in calendar days after sowing and then analysed in thermal time and photothermal time as potential ways of accounting for systematic variation in the response. Reanalysis of a dataset from the literature was used as validation of the thermal time to flowering theory. Because grazing management for seed production and re-establishment is crucial for continued balansa clover presence in a grazed sward, this chapter also defined visual, easily observable reproductive stages of balansa clover development. This generic development scale may be appropriate for use with other pasture legumes.

Thus, the objectives of the experiment described in this chapter were 1) to quantify the time to first flower from different sowing dates and 2) quantify the time of physiological seed maturity using environmental variables and then 3) relate that to a visual scale.

5.2 Materials and Methods

The experimental design was described in Chapter 4 (Experiment 2). Briefly, a split plot factorial in a randomised complete block was sown with main plots as sowing date and split plots as cultivar. Three replicates of two cultivars ('Bolta' and 'Frontier') of balansa clover were sown on eight dates (Table 4.2) at Lincoln University,

Canterbury, New Zealand. Plots were sown as pure swards and managed without grazing or cutting to give the maximum seed set possible.

5.2.1 Reproductive development

5.2.1.1 From vegetative to reproductive development

The appearance of reproductive structures was recorded on six marked plants per plot at weekly intervals from bud visible to seed shatter. The marked plants were the same as those used for vegetative development measures (Chapter 4; Experiment 2). The first three to five inflorescences per plant were marked as they appeared and were scored successively using the development scale described below. The analysis of the time of flowering was based on these data. Flowering was defined as when the first bud was visible on >50% of the plants surveyed/tagged and calculated from linear relationships between the mean number of plants with flowers present against the observation date. Full flower (Craig, 1998; Craig *et al.*, 2000) was defined as the time when maximum numbers of inflorescence were fully open within a plot.

Only 'Frontier' was measured from plants sown on 5 February 2007 (the 8th and last sowing date) because the experiment ended before 'Bolta' produced flowers due to paddock re-allocation.

5.2.1.1.1 Thermal and photothermal time

The time from emergence to flowering was defined using chronological, thermal and photothermal time calculations. Temperatures were recorded on-site at 1200 mm above ground level (air temperature), 100 mm above ground level and 10 mm below ground level (soil temperature) (Section 4.2.1.4.1). For all thermal time calculations, base temperature (T_{base}) was set at 2.5 °C and optimum temperature (T_{opt}) was set at 15 °C based on the cardinal temperatures for vegetative development (Chapter 4). Thermal time was accumulated daily, as described in Section 4.2.1.4.2.

Further analysis of the time to flowering for balansa clover was performed using photothermal time. Photothermal time modifies the amount of thermal time

accumulated with regards to the daily photoperiod (Section 2.6.1). The ability to unify the rate of leaf appearance has previously improved accuracy of crop simulation modelling (Jamieson *et al.*, 1995a).

Photothermal time was calculated using the following equations (Weir *et al.*, 1984):

Equation 5.1
$$Pt = FP \times Tt$$

Equation 5.2
$$FP = [(Pp - Pp_{base}) / (Pp_{opt} - Pp_{base})]$$

Where *FP* is a photoperiod modification factor, *Pp* is the photoperiod at emergence in hours, *Pp_{opt}* is the optimum *Pp* and the *Pp_{base}* is the base photoperiod. Daylength was taken at emergence from calculations of the rise and set of the sun (when the upper edge of the sun (disk) is on the horizon) for Lincoln, Canterbury, New Zealand (43° 38'S, 172° 28'E) in 2006 (Anonymous, 2008). The optimum Pp was set to 15.7 hours and the base Pp was set to 8.6 hours to correspond to the maximum and minimum photoperiods at the site.

5.2.1.2 Reproductive development scale

A numeric development scale was generated to describe the progress of the individual balansa clover inflorescences grown in the field from bud visible to seed shatter (Table 5.1). The scale was initially comprehensive to ensure all important visual development stages were captured. From this, the scale was then refined and simplified to reduce the amount of data collection required to describe the key development stages (Section 5.3.2.3; Refined development scale). Using the scale required a score to be given for each inflorescence on a plant. Unlike other crop-based descriptions, this scale tracked development of individual inflorescences, similar to the scale developed for Lupin (*Lupinus* spp.) by Dracup and Kirby (1996). Fehr *et al.* (1971) described reproductive development of soybean with regards to the length of the seed pod which grows to be in excess of 30 mm at times. This is impractical for pasture legumes like balansa clover in the field. However, the later stages that describe the change in colour of the seeds within pods as development progressed (Fehr *et al.*, 1971) were able to be integrated into the development scale.

Plate 5.1, Plate 5.2 and Plate 5.3 show 'Frontier' balansa clover inflorescences and their corresponding development scores. The scale was generated and refined over three seasons (2005-2007) with the initiation of flowering defined as the ability to visually identify a single floral bud emerging from the stipule (Thomas, 1979).

Table 5.1 Visual scale outlining the reproductive development of individual balansa clover inflorescences

- 1 The inflorescence bud is visible in the axil of a leaf
- 2 The peduncle is visible, the calyx is green (G or GY) and no corolla are visible
- 3 A single corolla is visible
- 4 >80% of flowers within the inflorescence have a visible corolla
- 5 Full flower - 100% of corolla have the standard unfolded from the wings
- 6 All flowers within inflorescence show browning as an indication of pollination¹
- 7 Abscission layer formed and florets have drooped downwards
- 8 Pods are visible within inflorescence
- 9 >50% of outer pedicles show red (R) colouring
- 10 50% of pods are red
- 11 100% of pods are red
- 12 50% of pods are yellow (2.5 Y (8/8 to 10) or 5Y (8/8 to 10))
- 13 100% of pods are yellow
- 14 First sign of seeds darkening (7.5 YR (6/8) to 5 YR (2/3))
- 15 100% of seeds are dark (7.5 YR (6/8) to 5 YR (2/3))
- 16 Seed releases upon touching pods

Note: Values within parentheses correspond to Munsell colour charts for plant tissues (1977).

¹Petals that were brown/wilted with age were not counted.

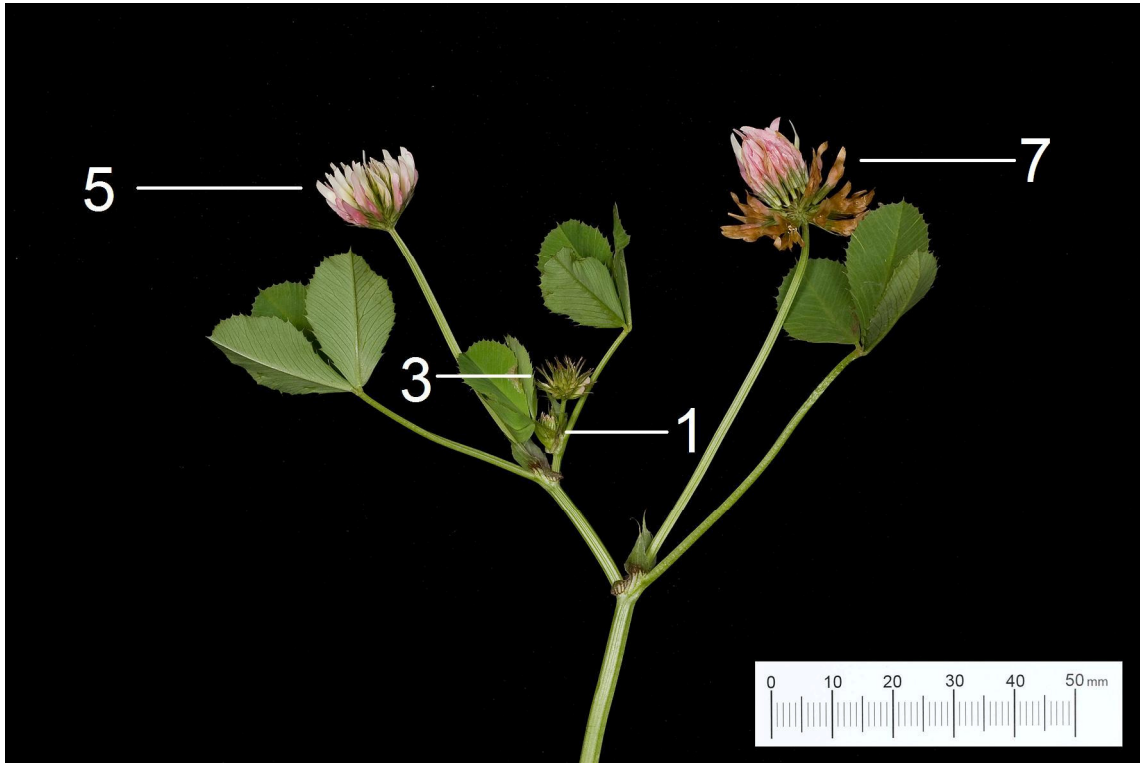


Plate 5.1 Inflorescence development scores for 'Frontier' balansa clover. Scores are presented in Table 5.1.



Plate 5.2 Inflorescence development for 'Frontier' balansa clover. Scores (Table 5.1) from left to right are: 1, 1, 2, 3, 4, 4, 5, 5, 7 and 7.



Plate 5.3 Inflorescence development for 'Frontier' balansa clover. Scores (Table 5.1) from left to right are: 8 and 9.

5.2.1.3 From full flower to physiological maturity

To record development of balansa clover inflorescences from full flower (stage 6) to seed pods bursting (stage 16), more detailed measurements were made. One hundred inflorescences per plot were marked from three sowing dates (27 April 2006, 30 August 2006 and 5 February 2007), shown in Table 5.2. Pollination was recorded as soon as any floret had turned from white to brown. Inflorescences were marked with a Max 'Tapener' HT-B2, fixed around the peduncle.

Table 5.2 Date that inflorescences were marked for detailed development observations at Iversen Field, Lincoln University, New Zealand.

Sowing Date	Date marked	
	'Bolta'	'Frontier'
27 Apr 2006	09 Nov 2006	13 Oct 2006
30 Aug 2006	18 Dec 2006	18 Dec 2006
5 Feb 2007	29 Mar 2007	29 Mar 2007

Note: All inflorescences were marked on the same day.

The three sowing dates were chosen because the first and third of them provided inflorescences that were formed approximately equal distance in chronological time from the second one, near the longest day (21 December). This was to account for

any differences that the daylength or direction of photoperiod change at flowering might have had on the time taken for seed maturity.

Stage 6 was chosen as the start point for detailed observations because it was field-observable. It is also beyond the point of influence of pollinator activity/inactivity, given the open pollination of this species. It allowed comparison with work already in the literature with white clover (Hyde *et al.*, 1959).

Every four days, starting on the day of marking, five of the 100 marked inflorescences were scored using the reproductive development scale, destructively harvested and dissected. Recording stopped when seed pods had shattered (Stage 16). The inflorescences from the 27 April 2006 sowing date were then dried at room temperature and weighed. No measure of seed moisture content was made – an oversight. Physiological maturity of the seed was defined as maximum dry weight of the inflorescence (Hyde, 1950) and moisture content was not considered important until it was too late to measure fresh weight. It was assumed that the increase in total inflorescence weight was reasonably related to the seed filling and that any decrease in dry weight (pod desiccation etc.) began at the time of seed maturity (Davies and Williams, 1986; Jamieson *et al.*, 2000).

5.3 Results

5.3.1 Time to flowering

5.3.1.1 Calendar days from emergence to flowering

The number of days from emergence to flowering (Stage 1) in 'Bolta' decreased with each successive sowing date through the year from 228 days when sown on 16 January to 58 days when sown on 1 December (Figure 5.1a). In contrast, the number of days from emergence to flowering increased for 'Frontier' from sowing on 16 January to 27 April and then decreased with each successive sowing after that (Figure 5.1b). The maximum number of days was 144 when sown on 27 April. The

minimum number of days was 42 ± 1 when sown on either 14 of October or 12 December.

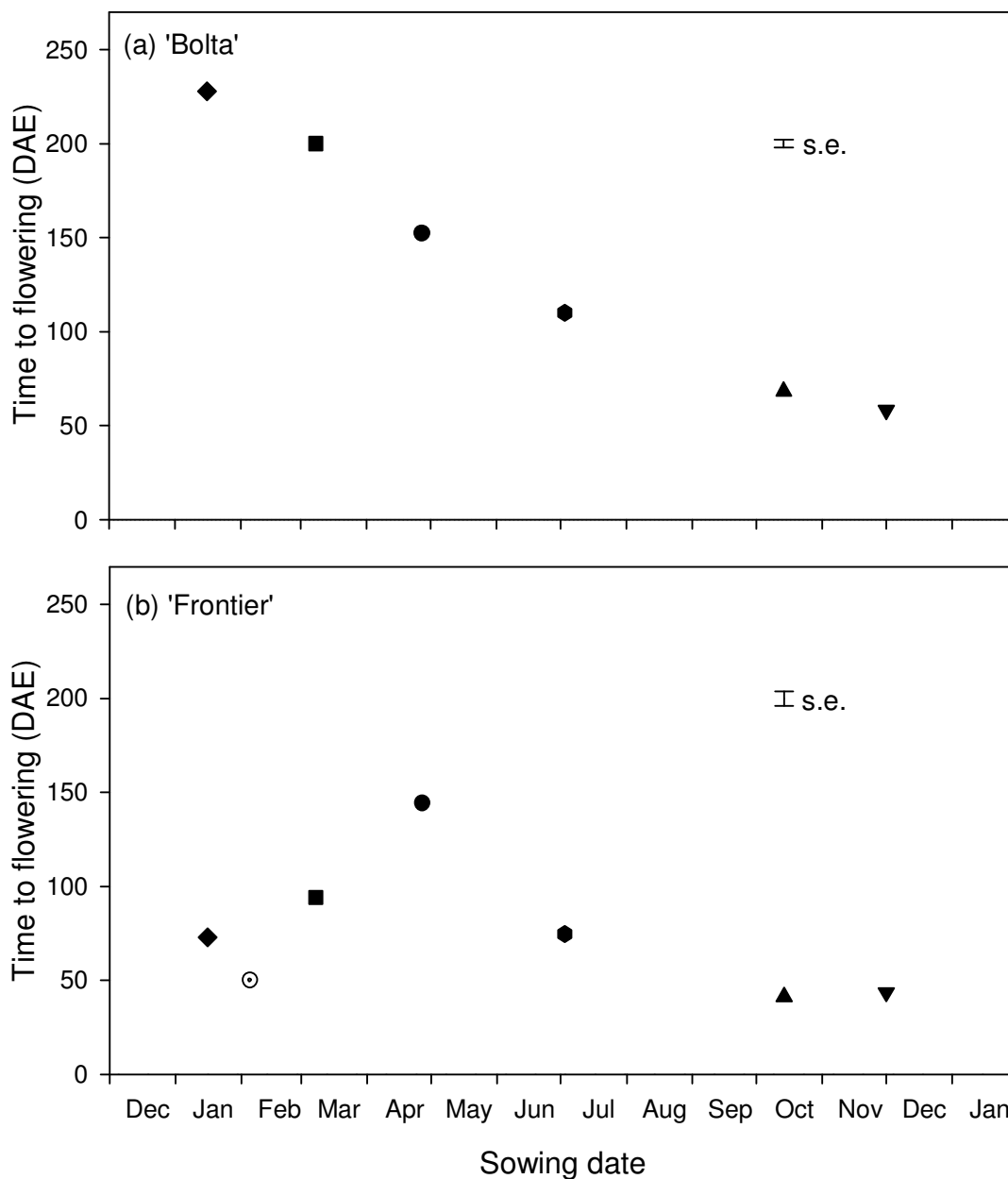


Figure 5.1 The number of days after emergence (DAE) to flowering for (a) 'Bolta' and (b) 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. 14 October 2005 (▲), 1 December 2005 (▼), 16 January 2006 (◆), 5 February 2007 (⊙), 8 March 2006 (■), 27 April 2006 (●), 3 July 2006 (◆).
 Note: s.e. is maximum standard error.

5.3.1.2 Thermal time from emergence to flowering

Thermal time was then calculated to try to explain the differences in calendar days from emergence to flowering (Stage 1) within each cultivar. The thermal time required ($T_{\text{base}} = 2.5 \text{ }^{\circ}\text{C}$) from sowing to flowering for 'Bolta' (Figure 5.2) exhibited a similar decline over time as seen for days (Figure 5.1). That is, thermal time ranged from a maximum of 1504 $^{\circ}\text{Cd}$ when sown on 16 January to a minimum of 575 $^{\circ}\text{Cd}$ when sown on 1 December.

When sown between 16 January and 27 April, the accumulated thermal time for 'Frontier' was ~690 $^{\circ}\text{Cd}$ from emergence to flowering (Figure 5.2). The thermal time then decreased stepwise to ~386 $^{\circ}\text{Cd}$ from sowing on 3 July through to 1 December. Thus there was not a constant total accumulated thermal time requirement for flowering for either cultivar. To account for the systematic variation in flowering date, the influence of photoperiod was examined.

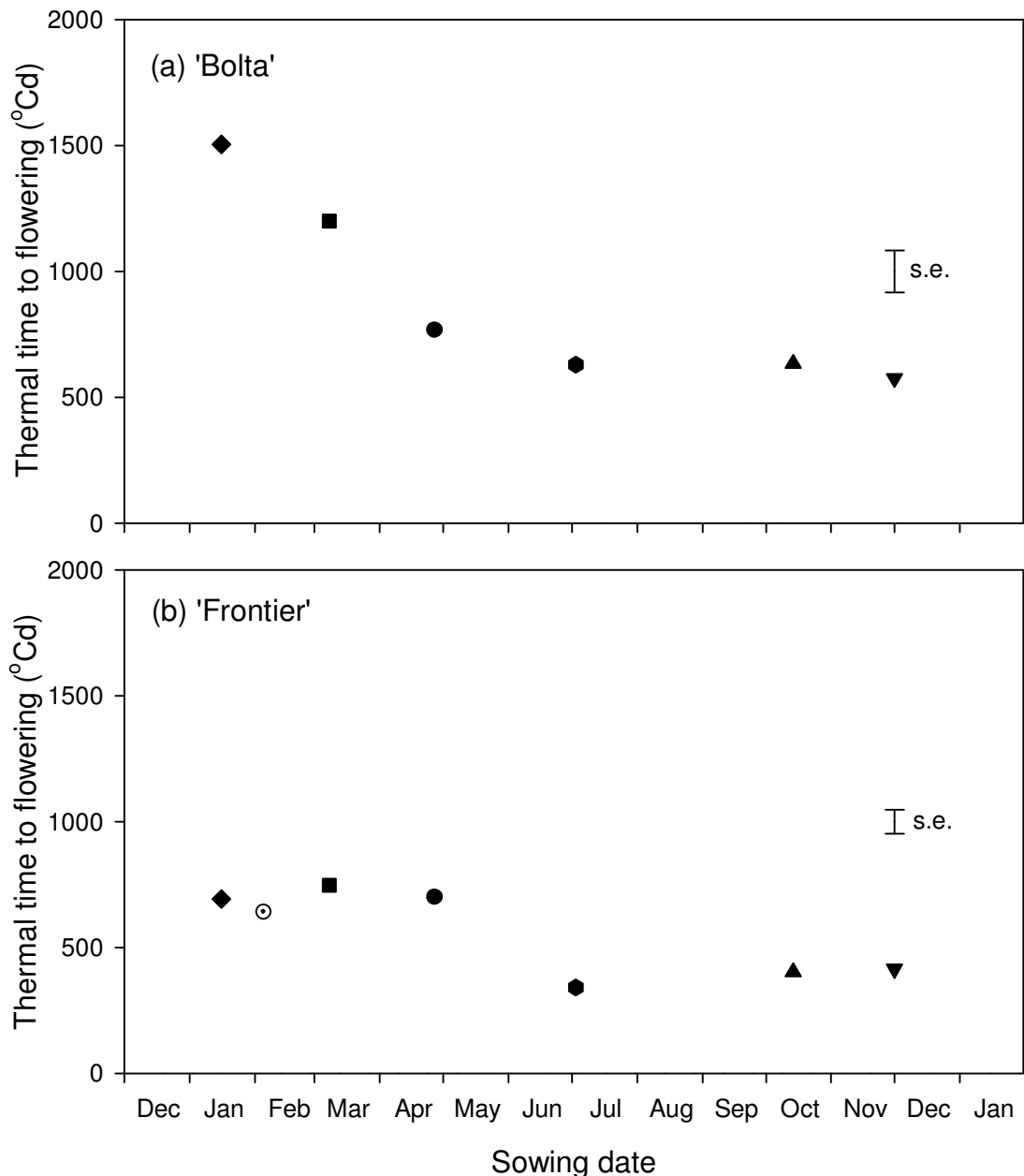


Figure 5.2 The number of thermal units from emergence to flowering for (a) 'Bolta' and (b) 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. 14 October (▲), 1 December (▼), 16 January (◆), 5 February (⊙), 8 March (■), 27 April (●), 3 July (◆). Note: $T_{base} = 2.5^{\circ}C$. s.e. is maximum standard error. For the year of sowing, see Table 4.2.

5.3.1.3 Photoperiod at emergence

Thermal time from emergence to flowering formed a hysteresis with increasing and decreasing Pp at emergence for both 'Bolta' and 'Frontier' (Figure 5.3).

5.3.1.3.1 'Bolta'

When 'Bolta' balansa clover seedlings emerged into decreasing photoperiods, the number of thermal units from sowing to flowering ranged from 1504 °Cd at a Pp of 15 hours (16 January) down to 629 °Cd at 9 hour Pp (3 July). This relationship showed that in decreasing photoperiod each additional hour of daylength at emergence raised the thermal time to flower by 147 °Cd (Equation 5.3). Standard errors are given in square parentheses.

Equation 5.3 $Tt = (148 [5.1]^\circ Cd \times Pp) - 716 [61.4]^\circ Cd \quad (R^2 = 100\%)$

Where Tt is thermal time (°Cd; T_{base} = 2.5 °C) and Pp is photoperiod (sun rise/sun set) in hours at emergence as a decimal.

'Bolta' balansa clover plants that emerged after the longest day flowered in September, when photoperiod was between 11 and 12.3 hours.

In contrast, for 'Bolta' balansa clover seedlings which emerged into increasing Pp (following the shortest day (21 June)), the number of thermal units from sowing to flowering decreased (Equation 5.4). However, the change in accumulated thermal units was only from 638 °Cd to 607 °Cd.

Equation 5.4 $Tt = (-8 [7.2]^\circ Cd \times Pp) + 707 [91.4]^\circ Cd \quad (R^2 = 52\%)$

5.3.1.3.2 'Frontier'

In 'Frontier' balansa clover seedlings, the number of thermal units from sowing to flowering plotted against Pp at emergence also formed two linear relationships. The relationships formed a hysteresis with increasing and decreasing Pp at emergence. When emerging into decreasing Pp, 'Frontier' plants took ~690 °Cd to flower as described in Equation 5.5. When emerging into increasing Pp, 'Frontier' took ~365 °Cd to flower (Equation 5.6).

Equation 5.5 $Tt = (-7 [13.2]^\circ Cd \times Pp) + 790 [175.1]^\circ Cd \quad (R^2 = 13\%)$

Equation 5.6

$$T_t = (12 [2.1]^\circ \text{Cd} \times P_p) + 234 [26.4]^\circ \text{Cd} \quad (R^2 = 97\%)$$

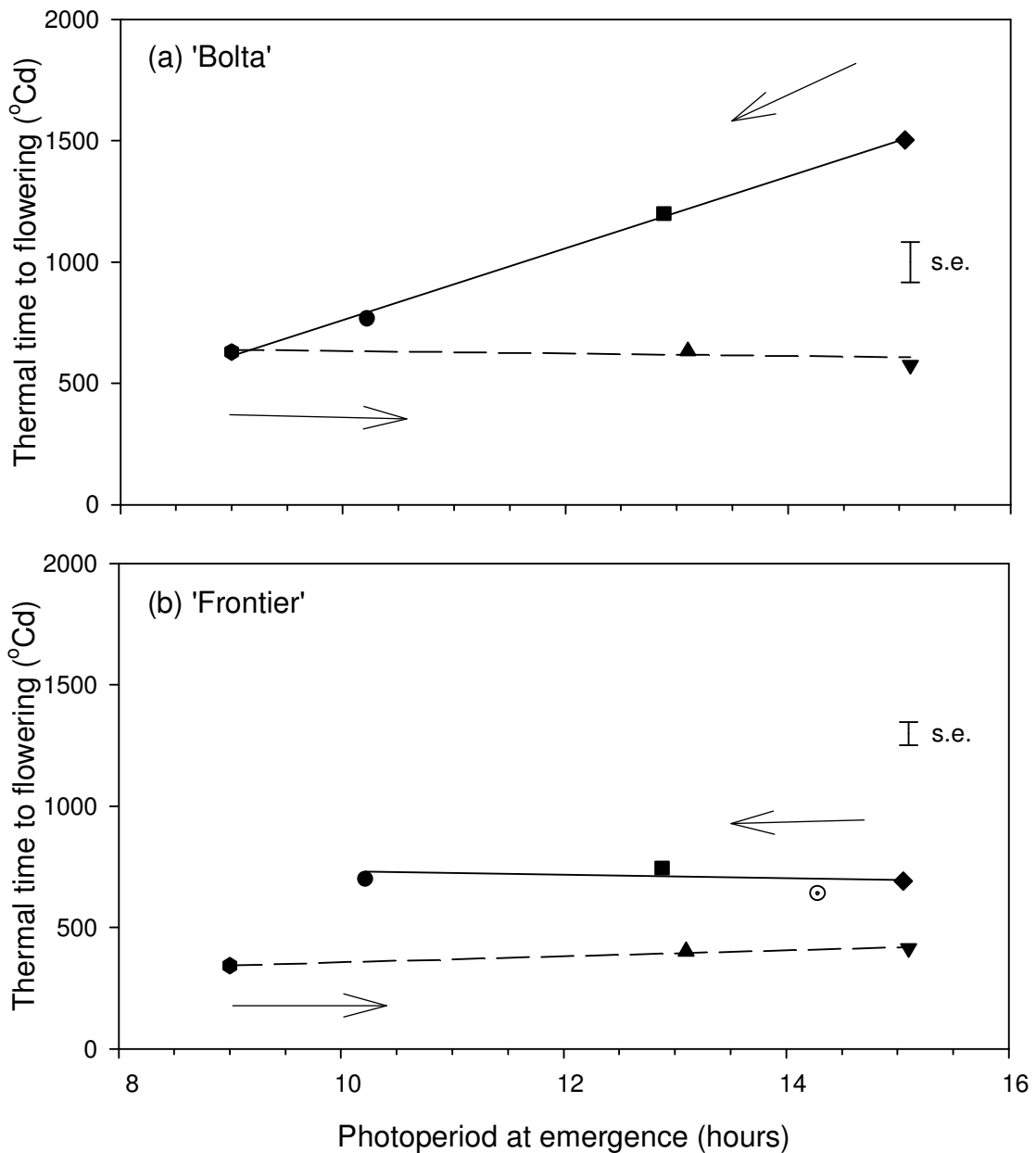


Figure 5.3 Thermal time from emergence to flowering against the photoperiod (sun rise/sun set) at emergence of (a) 'Bolta' and (b) 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. 14 October (▲), 1 December (▼), 16 January (◆), 5 February (⊙), 8 March (■), 27 April (●), 3 July (◆).

Note: Linear models are by least squares regression. Standard errors of the coefficients are presentment in Section 5.3.1.3. $T_{base} = 2.5^\circ \text{C}$, $T_{opt} = 15^\circ \text{C}$. s.e. is maximum standard error. For the year of sowing, see Table 4.2.

5.3.1.4 Photothermal time to flowering

The data were analysed further using photothermal time in an attempt to remove the hysteresis. By modifying the daily thermal time accumulation by photoperiod, the differences between times from emergence to flowering were reduced (Figure 5.4). Further analysis of the time of flowering against photoperiod at emergence confirmed this (Figure 5.5).

The photothermal time of flowering for 'Bolta' balansa clover was described ($R^2 = 79\%$) by the linear function:

Equation 5.7 $PTt = (64 [16.3] \text{ } ^\circ\text{Cd} \times Pp) - 362 [208.7] \text{ } ^\circ\text{Cd}$

The photothermal time of flowering for 'Frontier' balansa clover was described ($R^2 = 88\%$) by the linear function:

Equation 5.8 $PTt = (57 [9.5] \text{ } ^\circ\text{Cd} \times Pp) - 428 [124.6] \text{ } ^\circ\text{Cd}$

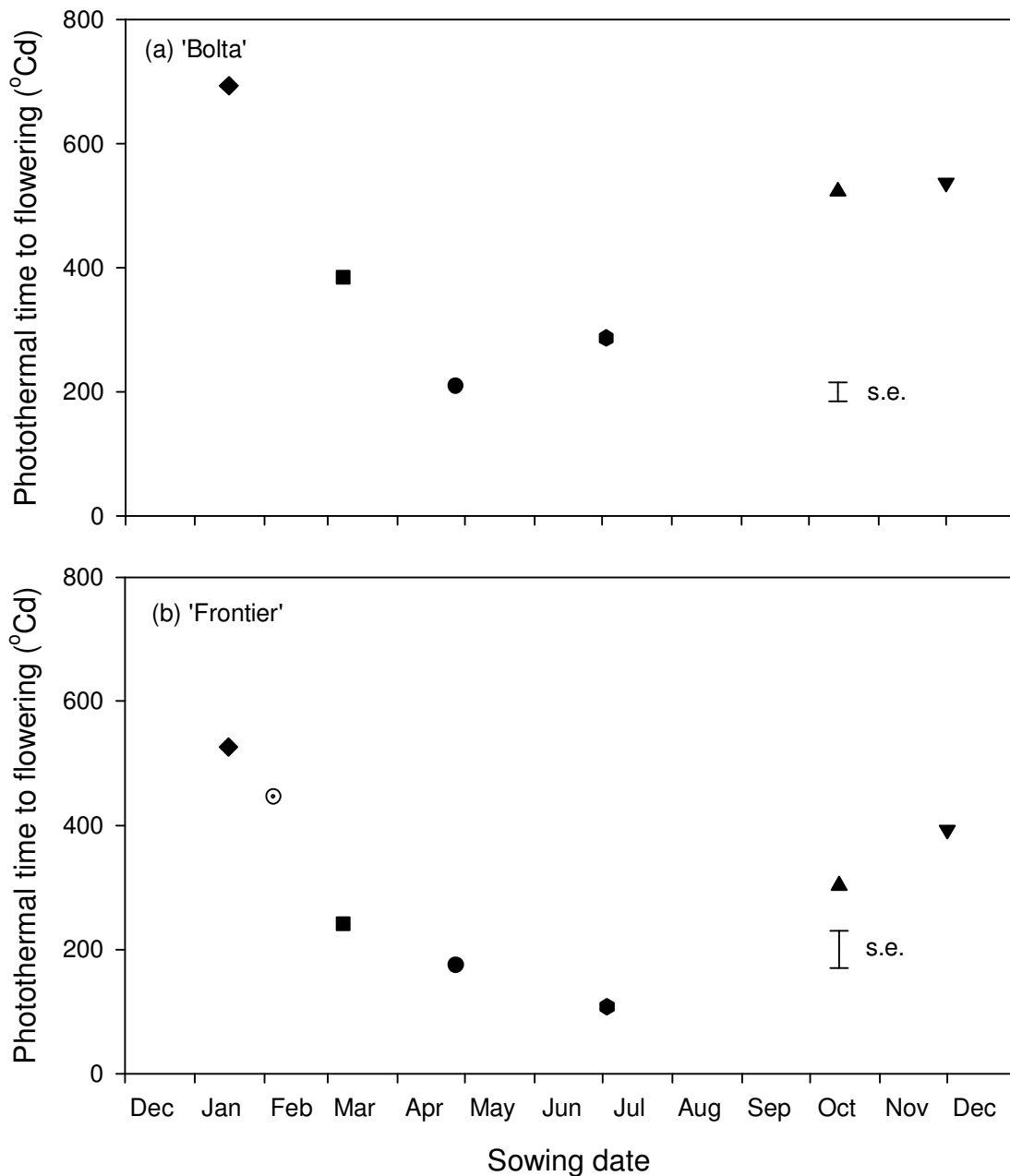


Figure 5.4 The photothermal time requirement from emergence to flowering of (a) 'Bolta' and (b) 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. 14 October (▲), 1 December (▼), 16 January (◆), 5 February (⊙), 8 March (■), 27 April (●), 3 July (◆). Note: $T_{base} = 2.5\text{ }^{\circ}\text{C}$, $T_{opt} = 15\text{ }^{\circ}\text{C}$. $Pp_{base} = 8.6$ hours, $Pp_{opt} = 15.7$ hours. s.e. are maximum standard errors. For the year of sowing, see Table 4.2.

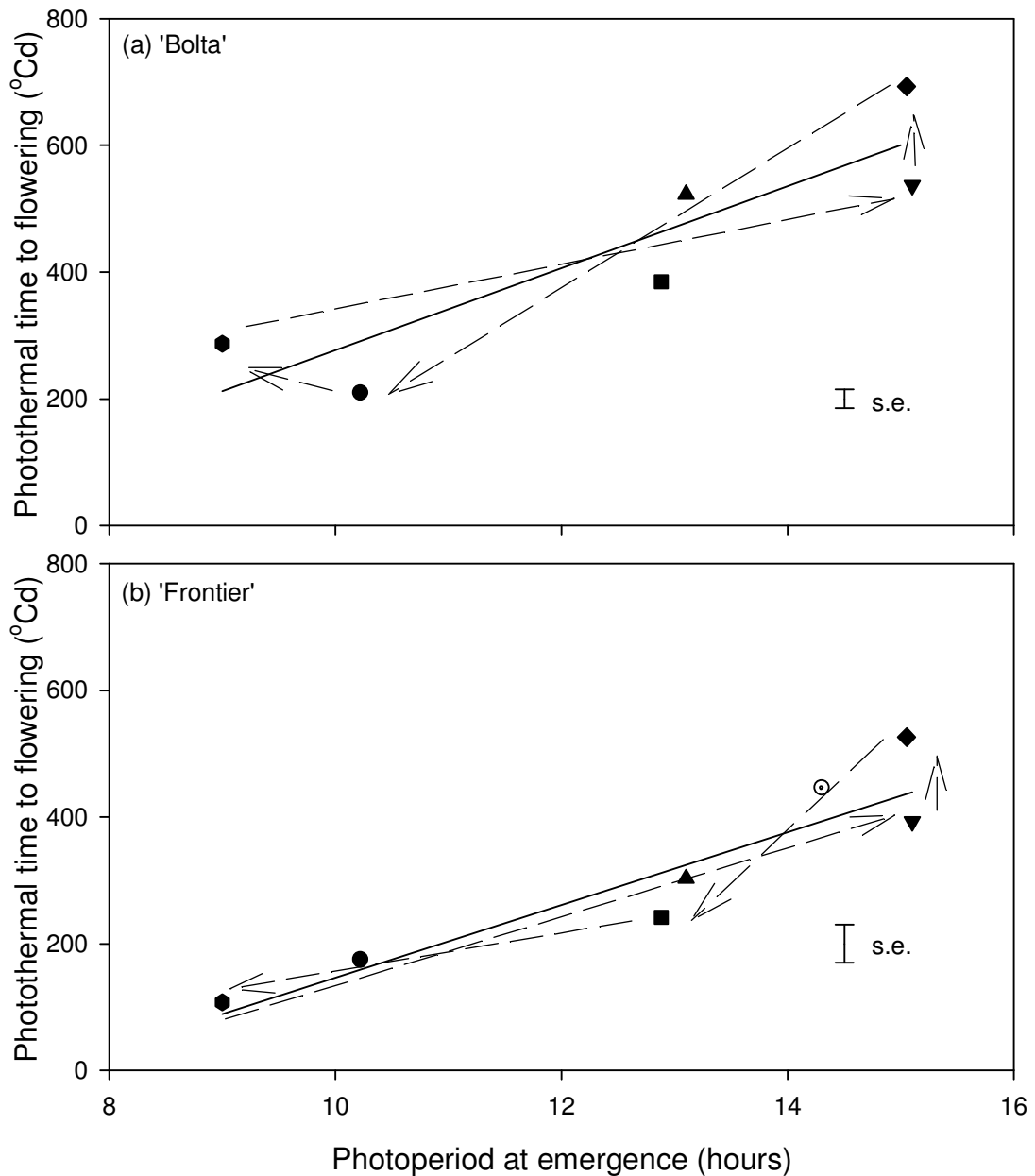


Figure 5.5 The photothermal time requirement from emergence to flowering of (a) 'Bolta' and (b) 'Frontier' balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. 14 October (▲), 1 December (▼), 16 January (◆), 15 February (⊙), 8 March (■), 27 April (●), 3 July (⬠).

Note: Lines show linear regression (—), and the direction of time (---), see text for equations.

$T_{\text{base}} = 2.5 \text{ }^{\circ}\text{C}$, $T_{\text{opt}} = 15 \text{ }^{\circ}\text{C}$. $Pp_{\text{base}} = 8.6 \text{ hours}$, $Pp_{\text{opt}} = 15.7 \text{ hours}$. Linear models by least squares regression. Coefficient of determination (R^2) and standard errors of coefficients are presented in Section 5.3.1.4. s.e. are maximum standard errors. For the year of sowing, see Table 4.2.

5.3.1.5 Reanalysis of Kelly and Mason (1986)

Thermal time from emergence to flowering was calculated for CPI45856 balansa clover sown in northern Victoria, Australia from five dates between 7 February and 20 June 1982 and plotted against the photoperiod at emergence (Figure 5.6). CPI45856 is a genetic parent of 'Paradana', itself a parent of 'Frontier', but exhibited the same response as 'Bolta'. The emergence dates meant that all CPI45856 plants emerged into decreasing photoperiods. They flowered between 20 and 28 September, when photoperiod was ~12 hours. As photoperiod at emergence decreased, so the thermal time requirement to flowering decreased.

This relationship showed that in decreasing photoperiod each additional hour of daylength at emergence raised the thermal time to flower by 239 °Cd (Equation 5.9). Standard errors are given in square parentheses.

Equation 5.9 $Tt = (239 [28.1] \text{ } ^\circ\text{Cd} \times Pp) - 1561 [336.6] \text{ } ^\circ\text{Cd}$ ($R^2 = 96\%$)

Where Tt is thermal time (°Cd; $T_{\text{base}} = 2.5 \text{ } ^\circ\text{C}$) and Pp is photoperiod (sun rise/sun set) in hours at emergence as a decimal.

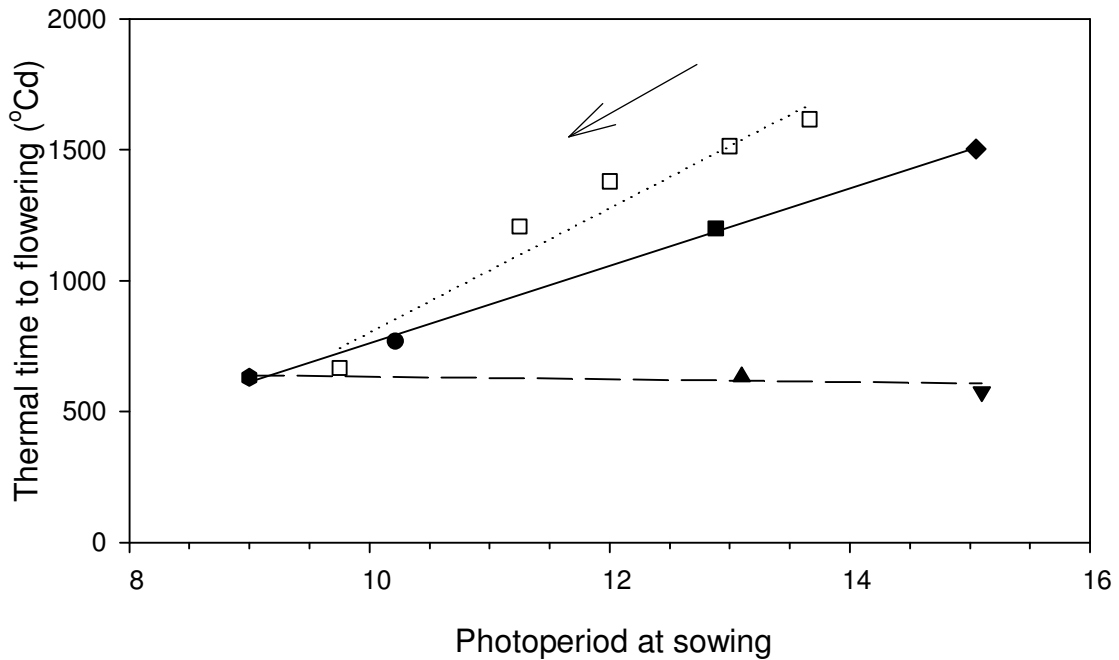


Figure 5.6 Thermal time from emergence to flowering against the photoperiod (sun rise/sun set) at emergence of 'Bolta' balansa clover (\blacktriangle \blacktriangledown \blacklozenge \blacksquare \bullet \blacklozenge , —, —) sown at different times at Lincoln University, New Zealand and CPI45856 (\square , \cdots) sown at different times in northern Victoria, Australia. Note: Linear models are by least squares regression. $T_{\text{base}} = 2.5\text{ }^{\circ}\text{C}$, $T_{\text{opt}} = 15\text{ }^{\circ}\text{C}$.

5.3.2 Development scale

5.3.2.1 The duration of an individual inflorescence

For 'Bolta' and 'Frontier' balansa clover sown in the field, inflorescences took between 42 and 52 days to progress from the first sign of pollination to stage 15, where all the seeds were dark within the pods (Figure 5.7). When analysed against thermal time ($T_{\text{base}} = 2.5\text{ }^{\circ}\text{C}$), this was about 350 °Cd for both 'Bolta' and 'Frontier' (Figure 5.8).

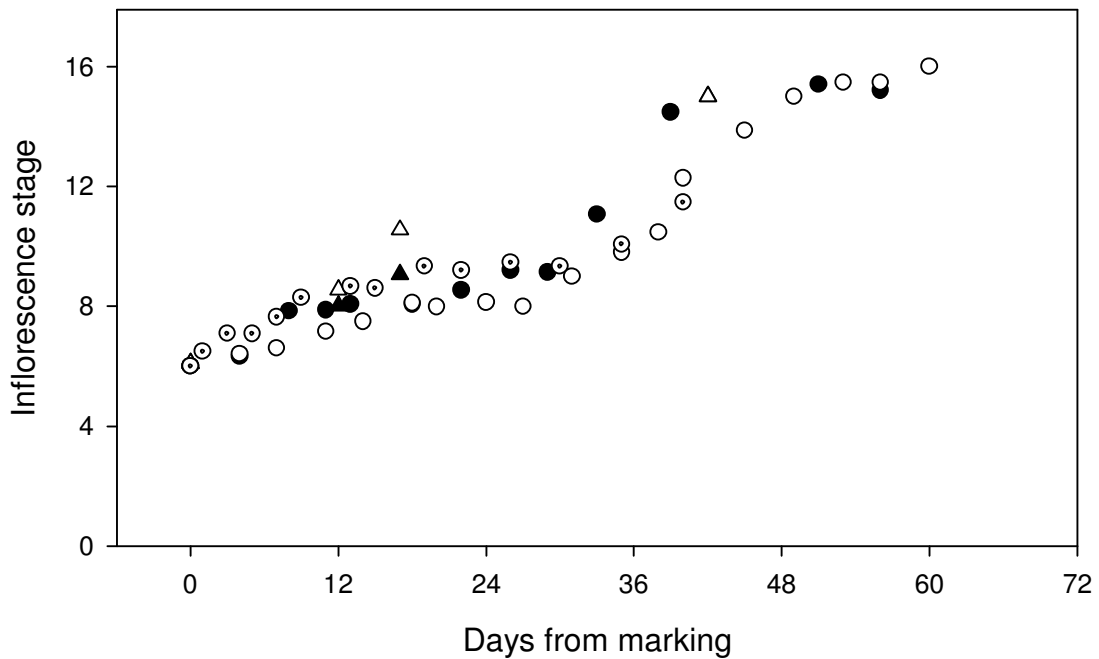


Figure 5.7 The inflorescence development stage of ‘Bolta’ (●,▲) and ‘Frontier’ (⊙,○,△) balansa clover against days from marking (Stage 6) sown at different times at Lincoln University, Canterbury, New Zealand. 5 February (⊙), 27 April (●,○) and 30 August (▲, △).
 Note: For the year of sowing, see Table 4.2.

5.3.2.2 Inflorescence weight as an indicator of seed maturity

Inflorescences that were marked on 13 October 2006, from plants sown on 27 April, reached their maximum weight after 34 days. Inflorescences of ‘Bolta’ and ‘Frontier’ reached their maximum weight at ~250 °Cd after marking (Figure 5.9). This point of maximum weight coincided with visual stage 10 (Table 5.1), when 50% of pods were red. This visual stage was, therefore considered an indicator of physiological maturity.

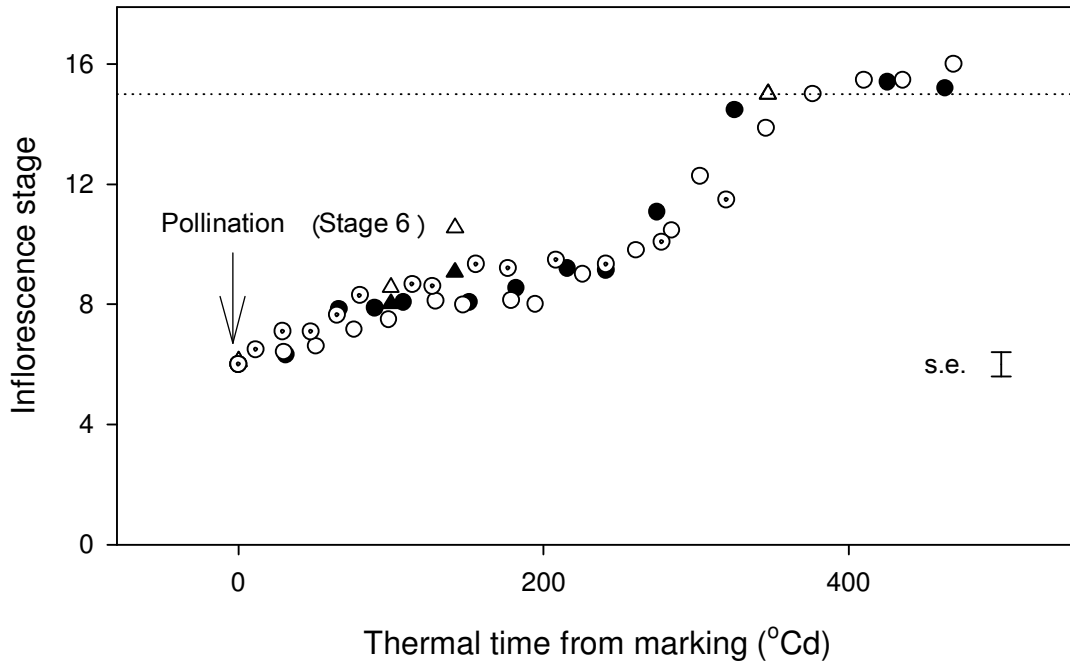


Figure 5.8 The inflorescence development stage of 'Bolta' (●,▲) and 'Frontier' (○,△) balansa clover against thermal time from marking (Stage 6) sown at different times at Lincoln University, Canterbury, New Zealand. 5 February (⊙), 27 April (●, ○) and 30 August (▲, △). ... is Stage 15, where all seeds are dark.

Note: $T_{\text{base}} = 2.5\text{ }^{\circ}\text{C}$, $T_{\text{opt}} = 15\text{ }^{\circ}\text{C}$. s.e. is the maximum standard error. For the year of sowing, see Table 4.2.

5.3.2.3 Refined development scale

The scale used for visual assessment of the inflorescence was refined once the raw data had been analysed. The revised scale (Table 5.3) focused on seed development and each of the five stages represents a logical step in that process. The first two stages describe vegetative growth, ending in a bud being visible in the axil of a leaf. The third stage, where all florets are fully expanded, indicated the inflorescence has reached full flower and approximates the time of pollination. This stage represented the earliest time that all florets were available to be pollinated. The fourth stage described the first visible seed pod within the inflorescence. The fifth and sixth stages describe when all seeds have transitioned from green and plump to dark and dry (Section 5.3.2.1).

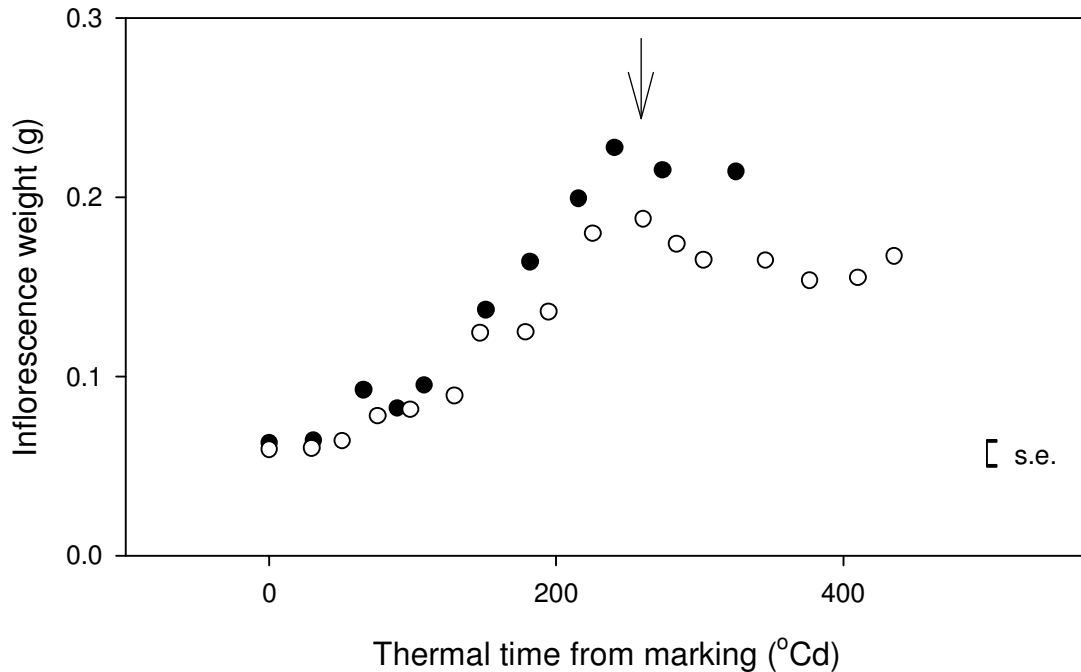


Figure 5.9 The average weight of individual inflorescences against thermal time from marking at Stage 6 for 'Bolta' (●) and 'Frontier' (○) balansa clover sown on 27 April 2006 at Lincoln University, Canterbury, New Zealand. Arrows indicate physiological maturity.

Note: Error bar is the maximum standard error of 0.09 g. $T_{base} = 2.5\text{ }^{\circ}\text{C}$.

Table 5.3 Field applicable visual scale outlining the development of individual balansa clover inflorescences

-
- 1 The plant has emerged
 - 2 Inflorescence bud is visible in the axil of a leaf
 - 3 Full flower - 100% of corolla have the standard unfolding from the wings
 - 4 Pods visible within inflorescence
 - 5 50% of pods are red (R)
 - 6 100% of seeds are dark (7.5 YR (6/8) to 5 YR (2/3))
-

Note: Values within parentheses correspond to Munsell colour charts for plant tissues (1977).

5.4 Discussion

5.4.1 Time of flowering

The time of flowering was measured in calendar days, thermal time and photothermal time. Both thermal time and photothermal time explained most of the variation in the time of floral appearance in balansa clover.

5.4.1.1 Thermal time

The time from emergence to flowering showed a distinct hysteresis based on whether the photoperiod at emergence was increasing or decreasing (Figure 5.3). For both 'Bolta' and 'Frontier', a plant which emerged into a decreasing photoperiod (21 December to 21 June) required more thermal time to flower than a plant which emerged into the same absolute daylength, but an increasing photoperiod (21 June to 21 December).

For each cultivar, linear equations quantified the duration between emergence and flowering for increasing and decreasing photoperiods. Aitken (1955) showed a response in subterranean clover flowering time to the photoperiod soon after emergence. It is feasible to expect a plant to be adapted to the direction of photoperiod change at the time of emergence. In the Mediterranean climate, where this plant germplasm was collected (Craig, 1998; Craig *et al.*, 2000), increasing photoperiods coincide with increased temperatures and decreased moisture availability. Photoperiod responsive pathways which induce flowering more swiftly (shorter thermal time) as Pp increases would be a positive survival mechanism. The consequence is a pre-disposition to produce seeds prior to death by drought. Plant survival, therefore, is promoted by the ability to produce reproductive structures sooner than would be necessary in more favourable conditions (Norman *et al.*, 2005). Natural selection would promote those plants that have the ability to differentiate between shortening or lengthening days as a surrogate for predicting future environmental conditions. Pollinating insects are also more likely to be active during the reasonably prolonged favourable conditions of spring (Herrera, 1990).

Jamieson *et al.* (1995a) promoted two hypotheses to explain differences in the timing of flowering in spring-sown wheat. These hypotheses state that the rate of leaf appearance is independent of daylength or its rate of change. In that work however the ultimate number of leaves, and therefore the timing of flowering, was determined by photoperiod 'some time after emergence'. The photosensitivity of plants has been shown to be higher before ontogenetic initiation of flowering than after (Roberts and Summerfield, 1987). However, because of the difficulties in identifying the specific

point in the field that photosensitivity changes, the level has typically been treated as remaining uniform from emergence until flowers appear. This assumption is not true for plants which exhibit a juvenile phase (Section 2.8.2). The critical period of photoperiod perception of the balansa clover plant may be during establishment, between emergence and appearance of the first trifoliate. Balansa clover, a dicotyledonous species does not contain the same leaf primordia which grass species typically have (Hartmann *et al.*, 2002). This, coupled with the relatively small size of the seed, may mean that balansa clover has a dramatically reduced leaf-production-burden before it is able to produce the first flower.

The two important elements of this theory could be tested conclusively using growth rooms. The first involves using contrasting constant photoperiods to determine the absolute short day/long day nature of the plant (Major, 1980). The second would involve reciprocal transfer experiments where plants are moved between short and long photoperiod environments to determine the timing and duration of photoperiod sensitivity (Adams *et al.*, 2001).

Using thermal time successfully described the time of flowering in 'Bolta' and 'Frontier' balansa clover in the field. It identified the shortcomings of using calendar days alone to predict reproductive development. It also showed cultivar-specific responses based on the photoperiod at the time of emergence. For both cultivars the thermal time requirement for flowering increased for seedlings which emerged into decreasing photoperiods (between 21 December and 21 June).

5.4.1.2 Photoperiod

When both 'Bolta' and CPI45856 balansa clover emerged into decreasing photoperiods, each flowered as daylength increased beyond 11.1 hours. 'Bolta' balansa, that emerged on 16 January 2006, flowered on 1 September when the photoperiod was 11.1 hours. CPI458567 sown on 7 February flowered first in northern Victoria, Australia on 20 September. Photoperiod at flowering was 12 hours. 'Bolta' and CPI45856 balansa clover that emerged after the longest day could be said to be long day plants (Section 2.8.2.1). Floral initiation began in 'Bolta' before 11.1 hours and in CPI45856 before 12 hours. Further work on the absolute

requirement for photoperiod induced flowering may give a greater understanding of the interaction between the direction of photoperiod change at emergence and the time to flowering. Dissection of floral primordia may also help form a stronger relationship between the time of flowering and the environmental stimuli.

5.4.1.3 Photothermal time

To unify the flowering time from any given sowing date based on the photoperiod at emergence, the amount of thermal time accumulated between emergence and flowering was modified by photoperiod. For an equation to account for the differences in flowering date from plants emerging on days with similar photoperiods, either side of the longest day e.g. 1 December and 1 January, the thermal time accumulated during that period must be accounted for. The photothermal time model does this by reducing the thermal time accumulated each day through the use of a base photoperiod, in this case 8.6 hours (Section 5.2.1.1.1). The relationship between photothermal time and the photoperiod at emergence quantified the time between emergence and flowering for 'Bolta' ($R^2 = 79\%$) and 'Frontier' ($R^2 = 83\%$) balansa clover in a single linear function (section 5.3.1.4). However, this approach did not satisfactorily account for the differences in flowering time when plants were sown before and after the longest day (Figure 5.5). Plants which emerged into similar absolute photoperiods on either side of the longest day (16 January and 1 December) had flowering times separated by 157 °Cd.

Analysis of the time of flowering was not improved by modifying the point at which the plant perceived the duration-defining photoperiod (Jamieson *et al.*, 1995a). These analyses included using the photoperiod at the first leaf, axillary leaf and first flower as the critical point of photoperiod duration perception. An example is given of the number of days after sowing for 'Bolta' balansa against the photoperiod at emergence and at 629 °Cd after emergence, the shortest amount of time between emergence and flowering (Appendix 17). This point was chosen as a possible indicator of the juvenile phase (Section 2.8.2). It was hoped by moving the time when the plant perceived daylength, the relationship between the time of flowering and absolute photoperiod might have been unified into one function for each cultivar. None of the changes in the date of photoperiod perception improved the relationship

beyond that of the photoperiod at emergence. It is therefore concluded the time to flowering was not determined by the absolute photoperiod, whenever it was perceived by the plant, but rather on the duration and direction of photoperiod. There appeared to be no juvenile phase in shoot apical meristem development for balansa clover.

Based on these analyses, thermal time was the most appropriate method of predicting the time to flowering. It appears that balansa clover set the amount of time required from emergence to flowering at emergence based on the duration and direction of photoperiod. This implies the plant perceived daylength, possibly by comparison of successive days or with an 'expectation' derived from an endogenous measure of circadian rhythm (Millar, 2004).

5.4.2 The lifespan of an individual inflorescence

For each cultivar, inflorescences from the three recorded sowing dates matured at a similar rate when measured in thermal time (Figure 5.8). When analysed against thermal time ($T_{\text{base}} = 2.5 \text{ }^{\circ}\text{C}$), the time from the first sign of pollination to maximum inflorescence weight, as an estimate of physiological maturity, was about 250 $^{\circ}\text{Cd}$ for both 'Bolta' and 'Frontier'. This compared with $\sim 390 \text{ }^{\circ}\text{Cd}$ ($T_{\text{base}} = 2.5 \text{ }^{\circ}\text{C}$) for white clover seed (Hyde *et al.*, 1959). These results confirmed previous work with annual legumes which show once an inflorescence was pollinated the rate of development depended solely on thermal time accumulation (Quinlivan *et al.*, 1987). The summer drought, which traditionally signals the end of the annual clover lifecycle in its native environment, may have led to the selection of plants that set seed in the least amount of time.

Physiological maturity occurred when inflorescences were at Stage 10, when 50% of pods were red. As inflorescence weight increased with time, the seed pod and seeds also changed colour. Because of this, the change of seed pod colour to yellow/red (within the range of 7.5 YR (6/8) to 5 YR (2/3) (Munsell, 1977)) could be used to indicate the timing of physiological maturity. Similar field-observable scales have been used to indicate maturity in soybean (Crookston and Hill, 1978) and canola (Elais and Copeland, 2001).

5.4.3 Grazing management

In Chapter 3, successful re-establishment of balansa clover required specific management for maximum seed set in the first season and subsequent top ups of the seed bank every four years. Chapter 4 showed balansa clover is a successful plant agronomically, with a comparable or faster rate of emergence and leaf appearance than subterranean clover. This chapter has shown sowing balansa clover soon after the longest day will increase the annual yield potential by lengthening the vegetative growing season. This work has reinforced the need to prepare the paddock for balansa clover emergence as soon as irrigation or autumn rains allow.

In addition, this work has shown once a flower has been pollinated, physiologically mature seed is present within the inflorescence after 250 °Cd (Figure 5.9). To a farmer this means that after the flower is pollinated seeds will be physiologically mature after two weeks at 20 °C air temperature. This gives the farmer the opportunity to control excess summer herbage through grazing at the right time without decreasing seed yield. It also allows a 100 °Cd (five 20 °C days) window for hay to be made where seed is physiologically mature and when the seed shatters from the pod (Stage 16) (Figure 5.8).

For a 'Bolta' balansa clover paddock which was established at Lincoln University, New Zealand on 8 March 2006, first flowers appeared on 24 September (Figure 5.1). Full flower is estimated to occur one month later on 24 October and contain physiologically mature seeds on 24 November. Hay could be cut until 6 December before seed begins to shed. With such high seed yields, there is the opportunity to remove half of the seed with a hay cut and to let half shatter.

Chapter 6 examines the timing and rate of germination of balansa clover in comparison with Persian clover, subterranean clover and white clover. It also quantifies the rate of recruitment of balansa clover seedlings in the field from the seed bank over two seasons and investigates hardseed breakdown in incubators.

5.5 Conclusions

The experiment described in this chapter quantified the timing of flowering for 'Bolta' and 'Frontier' balansa clover. Specific conclusions were:

- Plants which emerged into increasing photoperiods had a lower thermal time requirement for flowering than plants that emerged into the same, but decreasing, photoperiod. For example, 'Bolta' which emerged into a 13.1 hour increasing photoperiod required 630 °Cd to flower, compared with 1200 °Cd for plants which emerged into a 12.9 hour decreasing photoperiod.
 - The time from emergence to flowering was described using thermal time and was set within the plant by the photoperiod at emergence.
- Thermal time was used to describe the time between pollination and physiological maturity. 'Bolta' and 'Frontier' took ~250 °Cd ($T_{\text{base}} = 2.5\text{ °C}$). A further 100 °Cd was required for pods to burst and seeds to drop from the inflorescence. Physiological maturity coincided with 50% of seed pods turning a yellow/red colour.

6 Germination, recruitment and hardseed

6.1 Introduction

This chapter focuses on recruitment of seedlings from the seed bank. Chapters 4 and 5 were concerned with the rate of development of plants from seedlings to seed production and maturation. This chapter will show the rate and timing of germination for balansa, Persian, subterranean and white clovers at a range of temperatures in the incubator. A field experiment is used to show the amount and rate at which balansa clover seedlings are recruited from the seed bank each autumn for two years. Finally, another incubator experiment is used to show the response of hardseeded balansa clover seed to fluctuating temperatures.

The objectives of this chapter were firstly to define the cardinal temperatures and thermal time requirements for germination for balansa, subterranean and Persian clovers. The second objective was to determine the annual pattern of balansa clover seedling recruitment from the soil after an initial heavy seed set. The third objective was to show the response of balansa clover to classic techniques for softening hardseededness in growth rooms (Quinlivan, 1961, 1966).

6.2 Materials and methods

6.2.1 Experiment 8: Germination

For Experiment 8, three replicates of 50 scarified seeds per cultivar ('Bolta' and 'Frontier' balansa clover, 'Demand' white clover, 'Laser' and 'Nitro' Persian clover) were placed on moist blotting paper in petri dishes in unlit incubators (Sanyo MIR 512, Sanyo Electric Co., Japan) at 10 constant (24 hours) set temperatures, ranging from 5.0 to 35.0 °C at 5 °C intervals. Two other temperatures were added later, in an attempt to increase the number of data points below the optimum temperature for T_{base} analysis. Those temperatures were 12 and 22 °C. The actual temperatures achieved were recorded using a 'Hobo 4-Channel External' (Onset Computer Corporation) data logger at half hour intervals and these were integrated daily, and matched the temperature objectives. Seeds were scarified by rubbing between two

sheets of sandpaper (80 grit) until 5% of seeds had broken. No other pre-conditioning treatments were used. Distilled water was added as required to ensure moisture was non-limiting for germination. Petri dishes were re-randomised on a single incubator shelf after each count.

Germinated seeds were recorded and removed once or twice daily depending on the rate of germination until germination ceased. In legumes, germination was defined to be when the emerged radicle was twice the length of the maximum seed diameter. In grasses, germination was defined as the appearance of the coleoptile. Lonati *et al.* (2009) have since suggested germinated seeds should be counted when the radicle is the same length as the seed diameter. Imbibition was defined as the swelling of the seed to indicate the uptake of moisture.

White clover was chosen as the control because of its prominence in New Zealand agriculture (Caradus *et al.*, 1995) and to compare the results from this experiment with others in the literature e.g. Moot *et al.* (2000).

6.2.1.1 Data Analysis

Logistic curves (Equation 4.1) were fitted to the cumulative germination results to determine the final germination per cent and the number of days to reach 75% of the final germination. Cardinal temperatures were derived in the same way as presented in Section 4.2.3.

Data for each species were plotted as the reciprocal of the duration (in days) to 75% germination, where a linear relationship between this, the rate of germination, and the temperature indicated that the thermal time procedure was appropriate for the data.

6.2.2 Experiment 9: Seedling recruitment

For Experiment 9, seedling emergence was counted over two seasons from one replicated sowing date treatment sown on 16 January 2006 from Experiment 2 (Section 4.2.1). The replicated 'Bolta' and 'Frontier' monocultures were left ungrazed in 2006 to allow the maximum flowering and seed set. After seed had been shed

from the inflorescences, the remaining mature herbage was cut to a stubble height of 80 mm on 11 December 2006 using a rotary mower without the catcher. The mown material, which consisted of mostly stems, was then hand raked off the plots.

Rainfall between 20 December and 23 December 2006 of 56 mm (Figure 6.1) led to the germination and emergence of dense populations of balansa clover seedlings. Six 100 x 100 mm quadrats per plot were fixed to the soil on 5 January 2007 and seedlings were counted and removed. Subsequent re-counts were made from these quadrats following rain events through the summer/autumn until 10 May 2007. The experimental area was then knapsack sprayed with Roundup Transorb (glyphosate 540 g/L a.i) at 20 ml/L water on 22 May 2007 to kill the clover. This prevented flowering and seed production from the site in spring/summer 2007 (Year 2). Emergence counts from the seed set in 2006 continued after 56 mm of rain fell between 10 and 28 December 2007. Counting continued until 20 April 2008 when soil from fixed quadrats was dug out to a depth of 40 mm and any remaining balansa clover seed was hand sieved from the 36 soil samples.

6.2.2.1 Data analysis

Data from the recruitment experiment were analysed as a randomised complete block experiment by analysis of variance using Genstat Tenth Edition (10.1.0.72) (Lawes Agricultural Trust). All analyses were performed on untransformed plot data for each cultivar. Total estimated seed set was calculated as the cumulative number of seedlings removed from the fixed quadrats plus seeds remaining in the soil. In this experiment all seed was considered accounted for by either emergence or hardseededness with no allowance for predation or decay and none was observed at the experimental site. Seed losses in New South Wales have been reported to be as high as 50% (Jansen and Ison, 1995).

6.2.3 Experiment 10: Hardseed breakdown

Experiment 10 was a germination experiment to investigate the breakdown of hardseed in balansa clover. This experiment used non-scarified 'Frontier' balansa clover seed. The seed was harvested from mixed swards of cocksfoot and 'Frontier'

balansa clover grown at Ashley Dene Main Block paddock 8A on two dates (31 January 2007 and 1 March 2007) from the soil surface using a vacuum cleaner. The daily air and 100 mm soil temperatures between 1 January and 1 March 2007 are given in Figure 6.2.

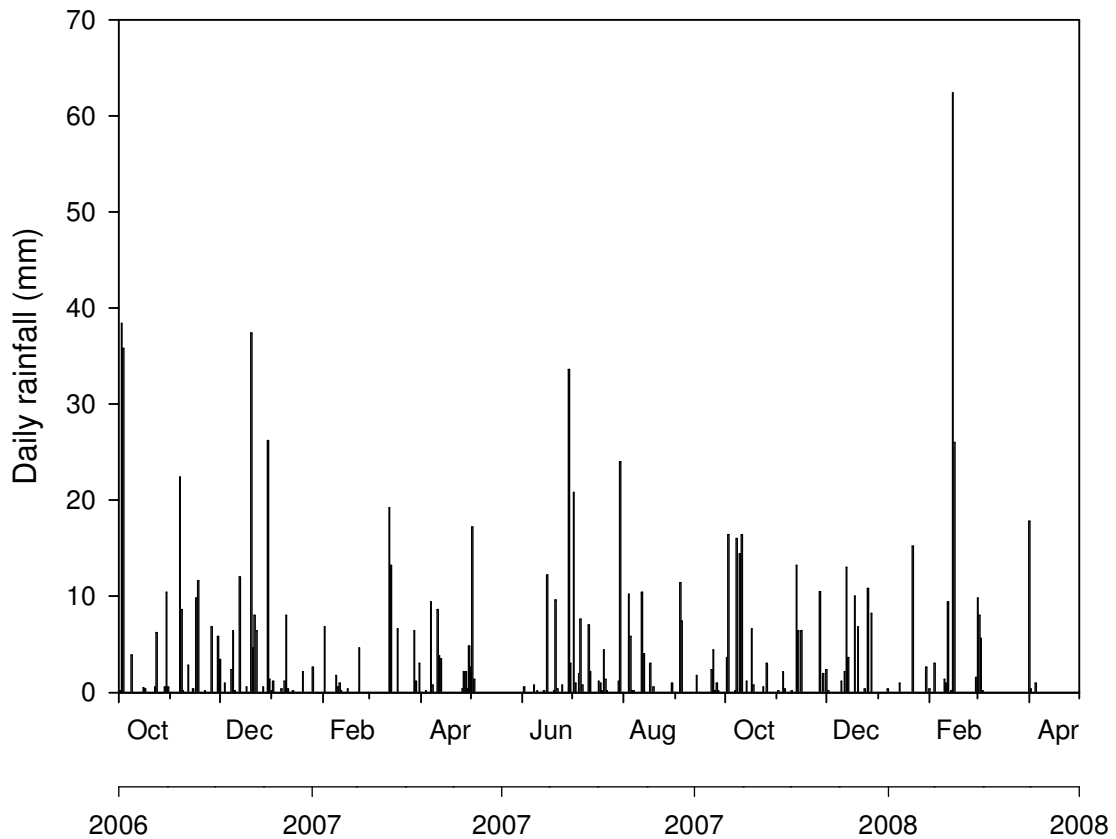


Figure 6.1 Daily rainfall from Broadfield Meteorological Station, ~2 km north of the experimental site at Lincoln University, Canterbury, New Zealand.

Seed was dressed using a Kumas Westrup seed cleaning machine and finished by hand cleaning. Seed was then stored at ambient temperatures in paper bags. Levels of hardseed were determined by germination tests on 12 July 2007 (133 days after the second harvest date) using five reps of 50 seeds of each of the seed lots in petri dishes on moist blotting paper at 12 °C for 10 days. The temperature and duration were selected because they were determined to be the species optimum for germination (Section 6.3.1).

Four treatments were then imposed in an attempt to break hardseededness: 12hrs each alternating (continuously) between 15 °C and 30, 40 or 50 °C (± 1 °C). A control

remained at ambient temperatures throughout the experimental period with temperature ranging from 10 to 22 °C. All treatments were imposed for periods of 54, 99 and 114 days. This gave 2 harvest dates x 4 temperatures x 3 time periods in a factorial design with 3 replicates. Seeds, including the control, were stored during treatment in dry, sealed, plastic petri dishes and temperature treatments were imposed using unlit incubators (Sanyo MIR 512, Sanyo Electric Co., Japan). After 114 days of treatment, the remaining seeds were moved (in petri dishes on moist blotting paper) into an incubator set to 12hrs each of alternating (continuously) 10 and 20 °C, to simulate autumn temperatures for 21 days.

Each seed lot was tested for viability by germinating 3 replicates of 50 hand-scarified seeds which had been stored at ambient temperatures from each harvest date on 3/11/07, 276 days after the first seed harvest. Seed was scarified between 2 sheets of 80 grit sandpaper.

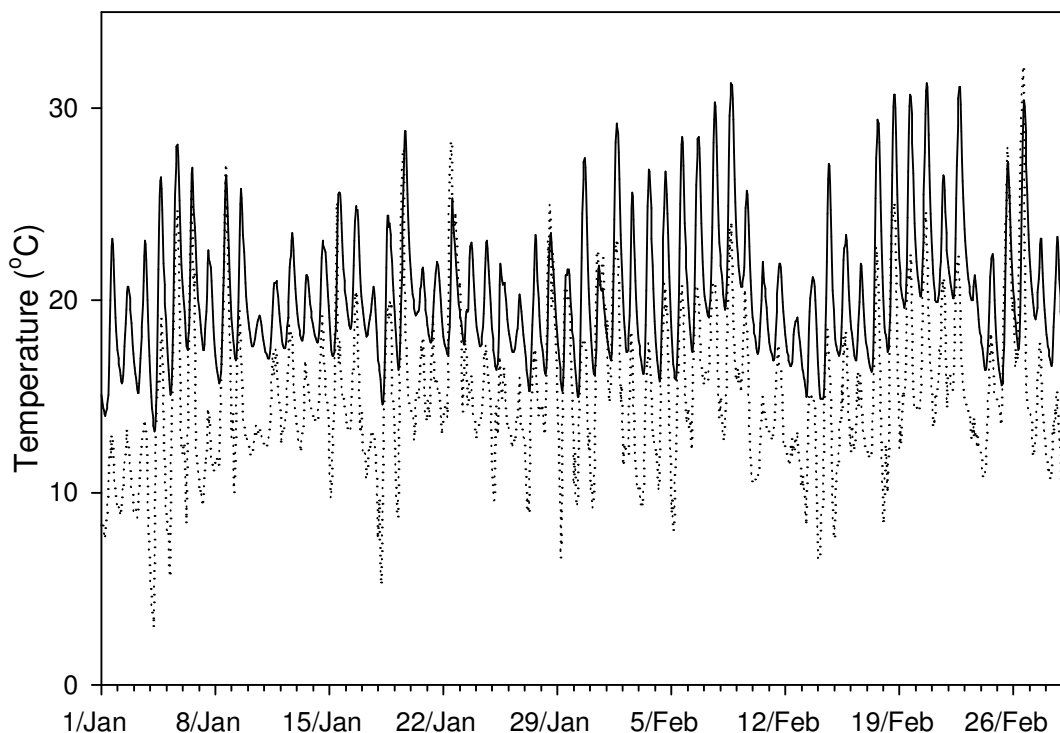


Figure 6.2 Air (···) and soil (100 mm) (—) temperature for 2007 recorded at Ashley Dene dryland research farm, Selwyn, Canterbury, New Zealand.

6.2.4 Experiment 11: Decreased rate of temperature change

Experiment 11 was a germination experiment following on from Experiment 10. One hundred seeds each of 'Frontier' balansa clover harvested from two dates (31 January 2007 and 1 March 2007) and held at room temperature until 26 February 2009 (Section 6.2.3) were subjected to fluctuating temperatures transitioning slowly over 12 hours between 15 and 30 °C (Figure 6.3). Germination percent was recorded after 7 and 14 days of treatment.

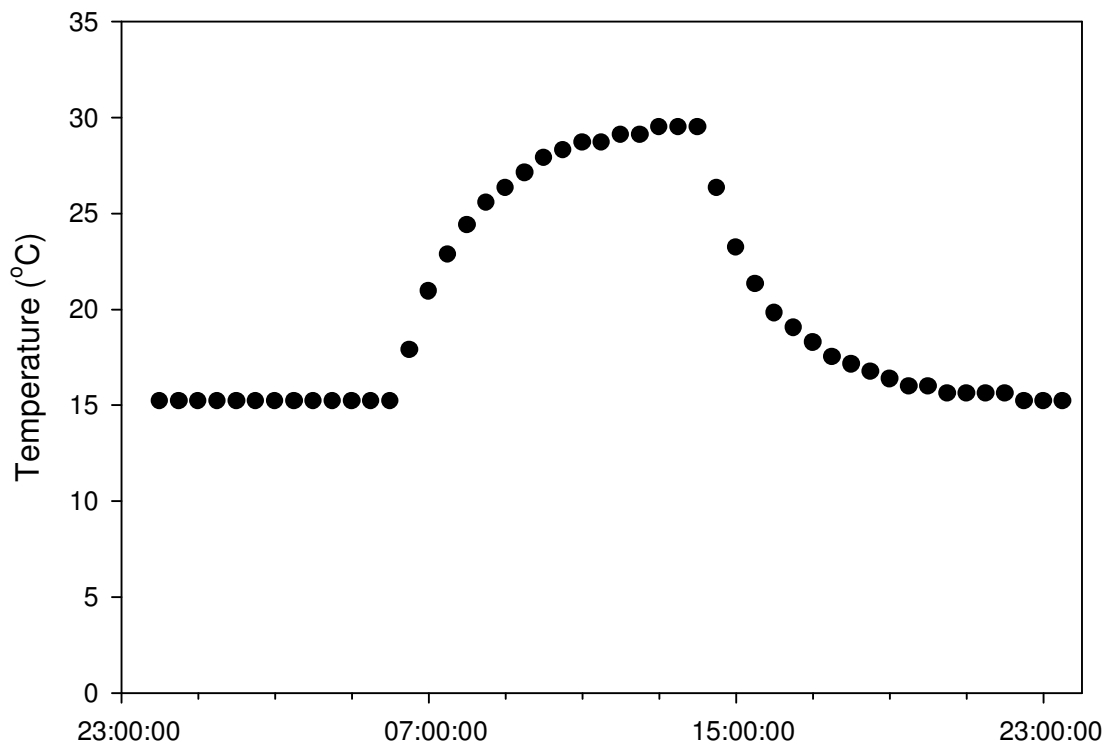


Figure 6.3 The temperature change in fine gravels placed in an incubator at Lincoln University, New Zealand used to moderate the temperature change in seeds.

6.3 Results

6.3.1 Seed weight

The seed weight was calculated from seed harvested from Experiment 2/9 for 'Bolta' as 1.17 mg and for 'Frontier' as 0.9 mg per seed.

6.3.2 Germination

For each cultivar, cumulative germination over time conformed to a generalised logistic curve. Few seeds populated the lower and upper regions of the curve, representing 'early' and 'late' germinating seeds (Figure 6.4). All seeds that imbibed ultimately germinated.

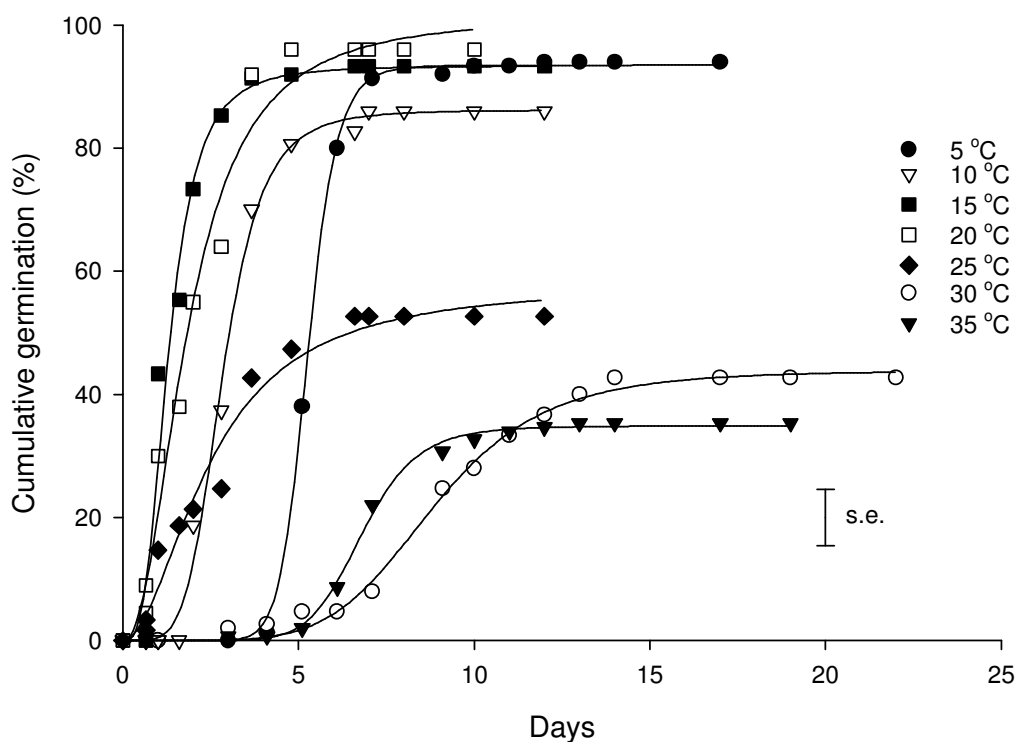


Figure 6.4 Cumulative germination of 'Bolta' balansa clover at different constant temperatures.

Note: s.e. is the maximum s.e. of means for the final germination percentage.

The rate and maximum cumulative germination percent differed with temperature for each species (Figure 6.5). For annual species, final germination percent remained constant between 5 and 20 °C and decreased to zero as temperatures increased

further. For example, final germination percent for 'Bolta' balansa clover ranged from 86 to 96% from 5 to 20 °C and then decreased steadily to 37% at 35 °C. The maximum germination percent for 'Laser' Persian clover was 64%. For 'Demand' white clover, final germination percent remained constant (~80%) across the whole temperature range and only declined at 35 °C.

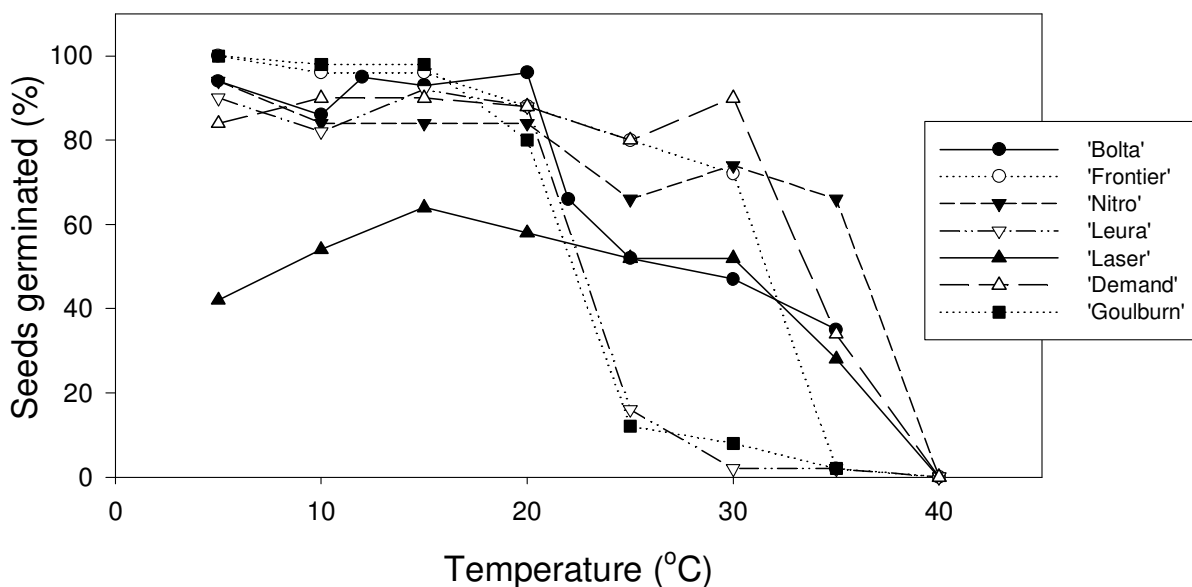


Figure 6.5 The maximum cumulative germination (%) of seeds for 'Bolta' and 'Frontier' balansa clover, 'Laser' and 'Nitro' Persian clover, 'Leura' and 'Goulburn' subterranean clover and 'Demand' white clover at different constant temperatures.

The mean number of days to reach 75% of maximum germination for each cultivar was affected by temperature (Appendix 18 and Appendix 19), typically decreasing as temperature increased toward ~15 °C and then increasing.

For 'Bolta' balansa clover the germination rate increased linearly from ~20% germination of seeds per day at 5 °C, up to ~55% per day at 15 °C (Figure 6.6 and Figure 6.7). Germination rate then decreased linearly to < 10% per day at 40 °C. This pattern of linear increase and decrease in the rate of germination ('broken stick') was uniform across cultivars and was used to define the cardinal temperatures (Table 6.1).

The base temperature for germination of 'Bolta' balansa clover was calculated to be 1.0 (± 4.9) °C; optimum temperature, 13.9 °C; maximum temperature, 40.2 (± 6.5) °C; and it required 26.3 °Cd to reach 75% germination at temperatures between T_{base} and T_{opt} ($R^2 = 95\%$). For 'Demand' White clover the cardinal temperatures were $T_{base} = -1.5$ (± 5.7) °C; $T_{opt} = 20.0$ °C; $T_{max} = 43.1$ (± 3.0) °C and $Tt = 49$ °Cd ($R^2 = 92\%$) (Table 6.1).

For all cultivars except 'Laser', 95% CI showed T_{base} estimates for germination were not different from 0 °C.

Table 6.1 Estimates of base (T_{base}), optimum (T_{opt}) and maximum (T_{max}) temperature and thermal time (Tt) for germination of a range of temperate climate legumes.

	T_{base} (°C) (†)	T_{opt}	T_{max} (†)	Tt (°Cd)	Tt °Cd ($T_{base}=0$ °C)
'Bolta' balansa clover	1.0 (± 4.9)	13.9	40.2 (± 6.5)	26.3	28.6
'Frontier' balansa clover	-0.7 (± 14.2)	12.1	42.4 (± 6.4)	29.8	31.4
'Laser' Persian clover	5.7 (± 4.0)	26.3	40.8 (± 4.0)	19.9	27.9
'Nitro' Persian clover	1.8 (± 16.4)	12.3	42.5 (± 18.6)	25.6	31.3
'Demand' white clover	-1.5 (± 5.7)	20.0	43.1 (± 3.0)	49.0	44.6
'Goulburn' subterranean clover	1.2 (± 4.9)	15.0	31.2 (± 9.6)	55.4	61.7
'Leura' subterranean clover	0.1 (± 3.9)	19.7	31.0 (± 13.4)	61.2	61.7

Note: Potential temperature range was 5, 10, 12, 15, 20, 22, 25, 30, 35 and 40 °C, giving $n = 10$ when all temperatures were included in the regression (12 and 22 °C excluded for 'Demand', 'Leura' and 'Goulburn'; $n = 8$). Coefficients are given in Appendix 20. † is 95% confidence interval.

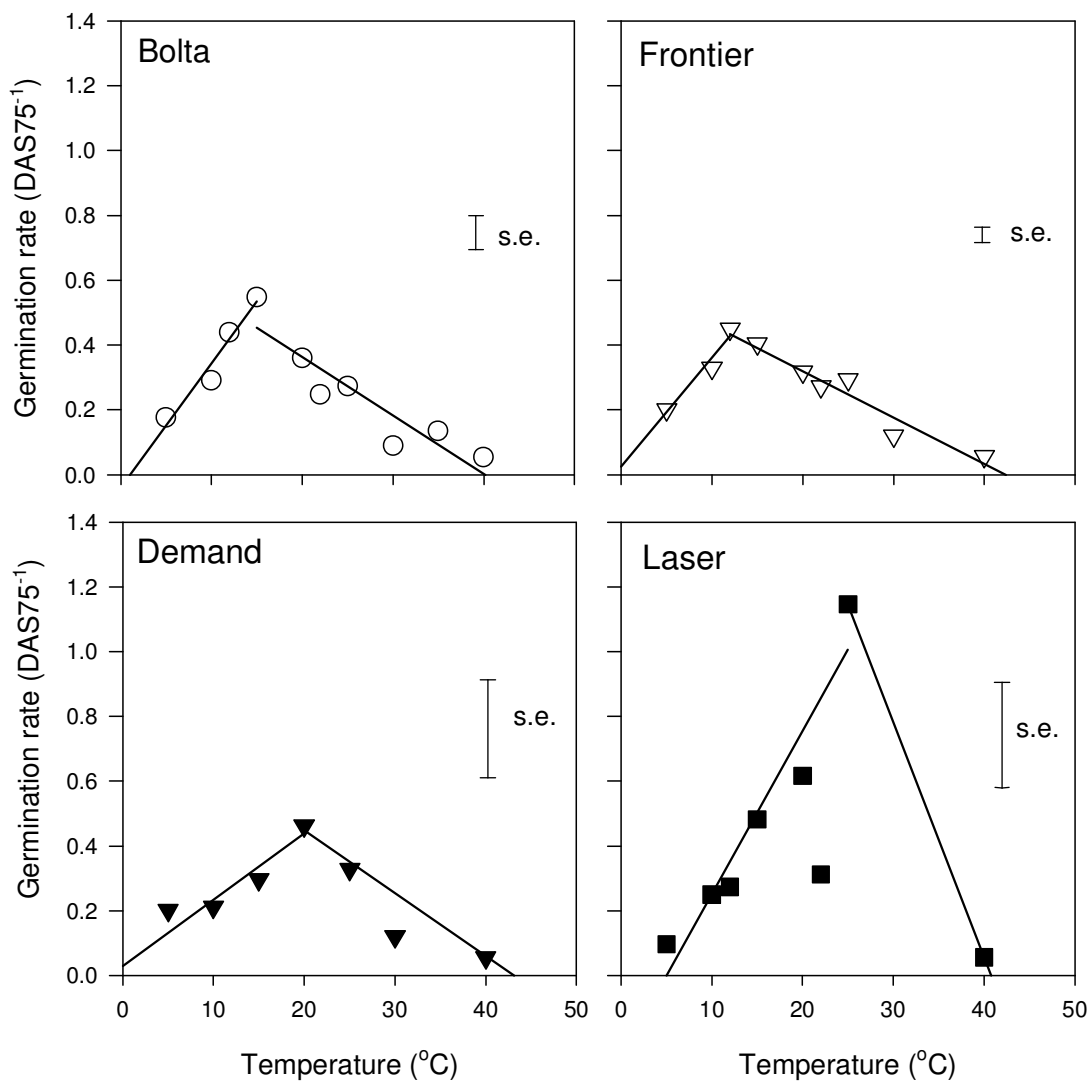


Figure 6.6 The rate of germination for 'Bolta' and 'Frontier' balansa clover, 'Demand' white clover and 'Laser' Persian clover at different temperatures.
 Note: s.e. are maximum standard errors of the mean. Coefficients are given in Appendix 20.

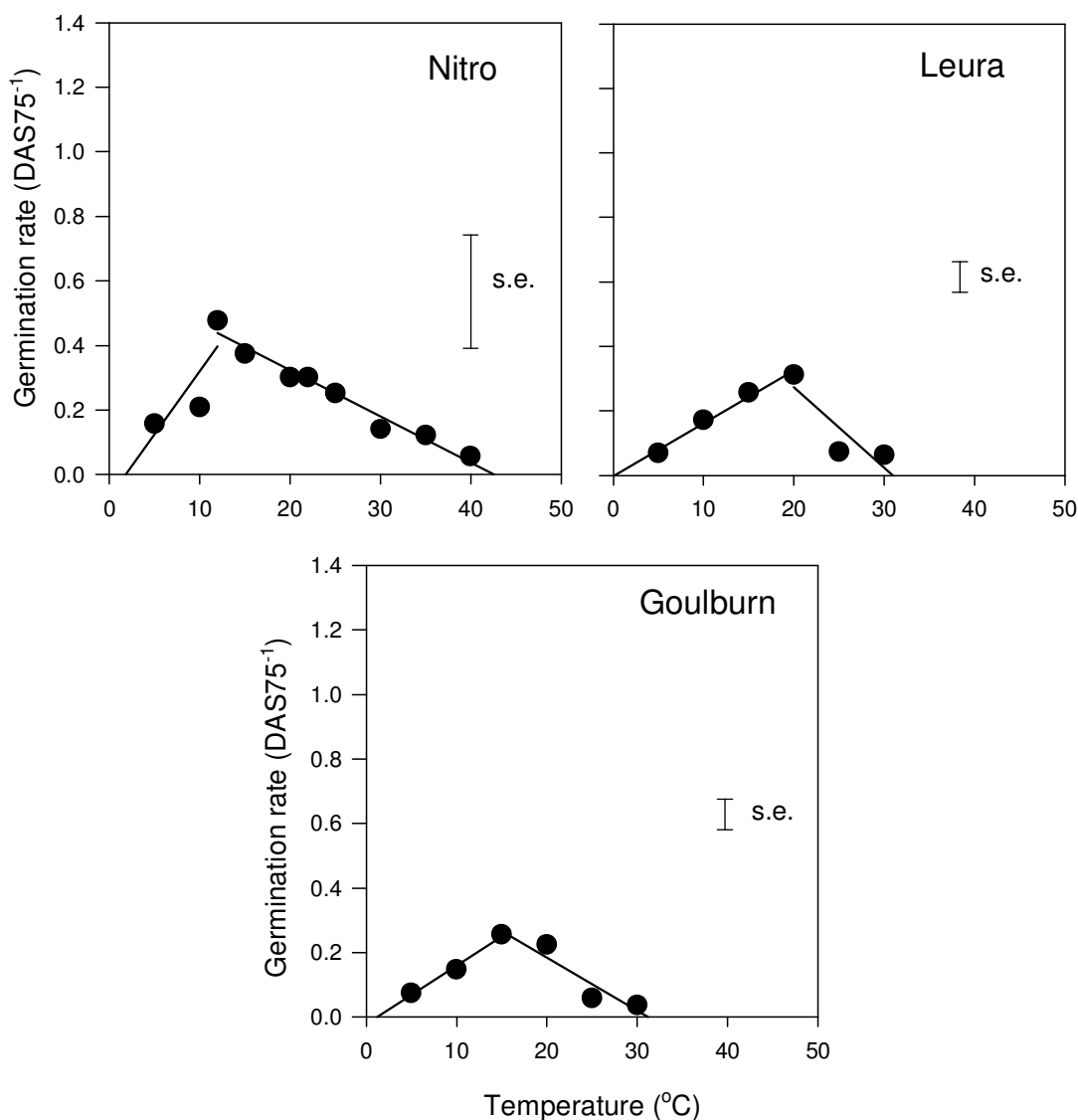


Figure 6.7 The rate of germination for 'Nitro' Persian clover and 'Leura' and 'Goulburn' subterranean clover at different temperatures.

Note: s.e. are maximum standard errors of the mean. Coefficients are given in Appendix 20.

6.3.3 Seedling recruitment

On 5 January 2007, 52% of 'Bolta' and 43% of 'Frontier' balansa clover seeds in the soil had produced seedlings (Figure 6.8), which averaged 24,000/m². By 16 January 2007, 65% of 'Bolta' and 54% of 'Frontier' seed had produced seedlings. Emergence counts ceased on 10 May 2007 with 27% of 'Bolta' and 37% of 'Frontier' seed assumed to remain hard. Emergence counts began again on 4 January 2008 and followed a similar pattern of seed softening to that seen in 2007. The total seedlings emerged and seed remaining in the soil at 20 April 2008 equated to ~50,000

seeds/m², or an estimated 600 kg seed/ha, produced in spring 2006. Thus, approximately 11% of the seed population that was set in November 2006 remained hard in April 2008 for both cultivars.

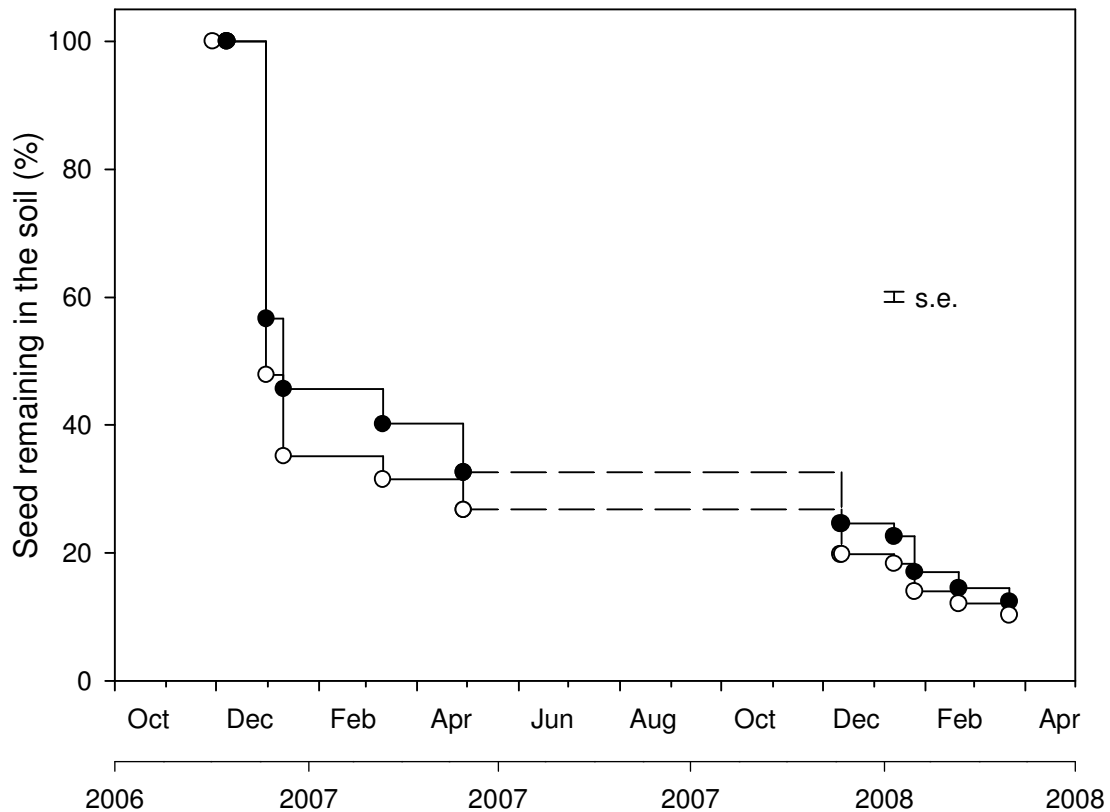


Figure 6.8 The percentage of 'Bolta' (●) and 'Frontier' (○) balansa clover seeds remaining in the soil after emergence and removal following rain events at Lincoln University, New Zealand.

Note: s.e. is the standard error of the seeds remaining in the soil (%) of 1.61.

6.3.4 Breakdown of hardseed

'Frontier' balansa clover collected from Ashley Dene on 31 January 2007 and 1 March 2007 had 98% germination after seeds were scarified on 3 November 2007.

Non-scarified 'Frontier' had a mean germination of 7.4% at the first germination (12 July 2007) with no difference between seeds harvested on different dates (Figure 6.9). On 3 November 2007, germination percent of seed harvested on 31 January 2007 had not changed. Germination percent of seed harvested on 1 March 2007 had increased to 20.1%. Fluctuating temperature treatments for up to 114 days resulted

in no significant variation in germination from the control for either harvest date. The 'autumn' temperature treatment that was imposed on seeds for a further 21 days also did not result in any change in the germination.

Seeds subjected to a slower temperature change between 15 and 30 °C in Experiment 11 did not exhibit any increase in the rate of hardseed break down. One seed in fifty germinated after 14 days of fluctuating temperature treatment from both harvest dates. Scarified seed germination at the same time was 98% in both seed lines.

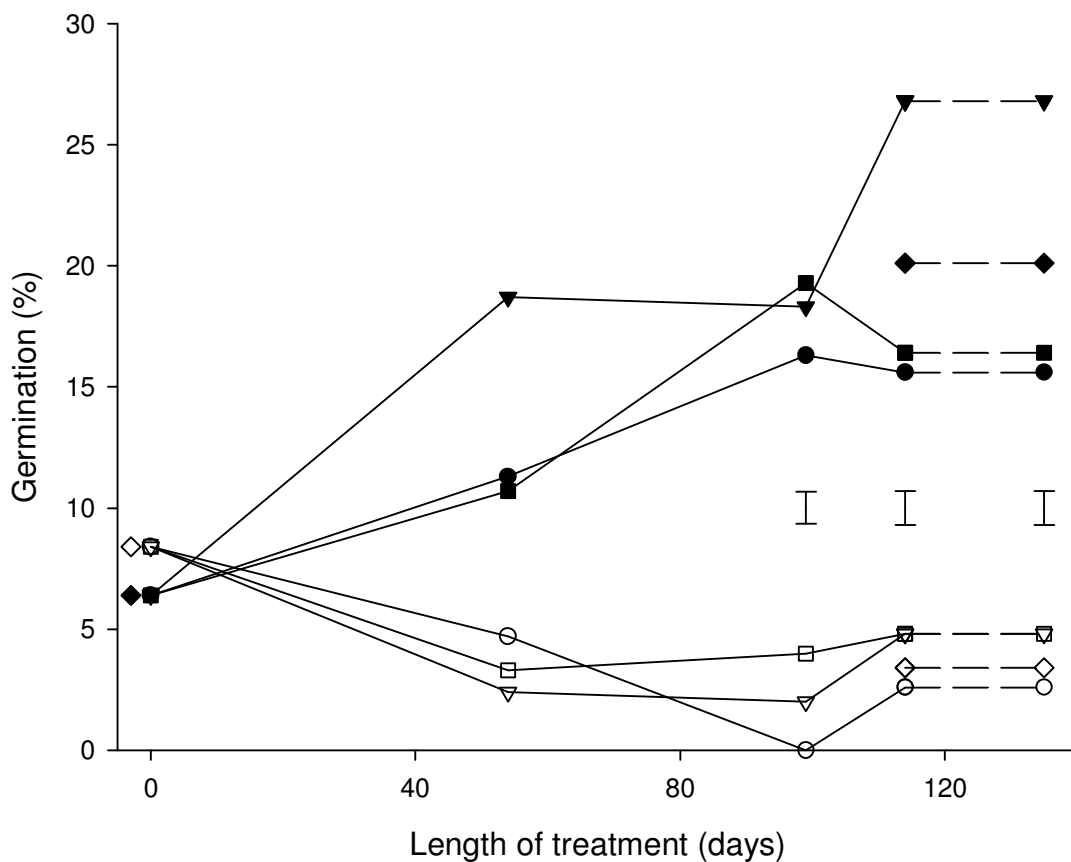


Figure 6.9 Germination percentage (%) of 'Frontier' balansa clover under 4 temperature treatments, 15/30 °C (●,○), 15/40 °C (▼,▽), 15/50 °C (■,□) and ambient temperatures (◆,◇) from harvests on two dates (31 January 2007 (○,▽,□,◇) and 1 March 2007 (●,▼,■,◆)) from Ashley Dene, Canterbury, New Zealand. Dashed lines (--) indicate germination after a further 21 days at 10/20 °C following germination on 114 days of treatment.

Note: Error bars represent standard errors for harvest date when P < 0.001.

6.4 Discussion

6.4.1 Germination

The germination rate of each species increased linearly as temperature rose from T_{base} until reaching a maximum at T_o (Figure 6.6 and Figure 6.7). The thermal time concept allows the time from sowing to germination at temperatures between T_{base} and T_{opt} to be expressed as a single coefficient. Base temperatures for all species were ≤ 5.7 °C and, except for 'Laser' Persian clover, none were different from 0 °C (Table 6.1). This and previous work (Moot *et al.*, 2000; Lonati *et al.*, 2009) suggests that, without evidence to the contrary, future work with annual pasture legumes species could assume $T_{\text{base}} = 0$ °C for germination.

As temperatures rose above T_o , germination rate decreased linearly until becoming zero at T_m . All species showed a similar response to temperature and the true sigmoid shape of the relationship at temperatures between T_{base} and T_{opt} was not seen (Angus *et al.*, 1981). The strong linear relationships confirm the appropriateness of using the linear response models when estimating thermal time (Lonati *et al.*, 2009). It was unexpected to see differences in the germination rate between 'Laser' and 'Nitro' Persian clover (Figure 6.6 and Figure 6.7). Germination rate is generally considered to be genetically determined and, without specific selection pressure, uniform within a species (Moot *et al.*, 2000). However, these cultivars may be different sub-species. 'Nitro' Persian clover had a maximum germination rate of 0.47 and is derived from the prostrate, hardseeded sub-species *resupinatum* (Wurst *et al.*, 2004). Other cultivars from this sub-species include 'Prolific' and 'Kyambro'. 'Laser' had a maximum germination rate of 1.2 and is believed to have been selected from the erect, later flowering subspecies, *majus*. Other cultivars from this sub-species include 'Lightning', 'Maral' and 'Mihi'. 'Mihi' has shown a similar germination rate to 'Laser', with comparable cardinal temperatures and thermal time requirements (Monks *et al.*, 2009). Future Persian clover cultivar development could, therefore, include improved germination rate as a selection criteria, especially for slow establishing species.

The typical lifecycle of these annual legumes starts with germination in autumn as moisture increases. Vegetative growth continues through winter and spring before

reproductive structures are formed and seed is set in spring/summer. Annual legumes avoid the drought conditions of their native environment as seeds (Sulas *et al.*, 2000). The decline in germination percent at higher temperatures ($>20^{\circ}\text{C}$) (Figure 6.5) would therefore limit seedling losses to out of season germination (false break) when moisture is insufficient to sustain growth (Cocks, 1996). This adaptation is referred to as high temperature dormancy and is a desirable characteristic in native populations (Knight, 1965). High temperature dormancy plays less of a role in preventing germination in New Zealand than in Australia or the Mediterranean, where summer temperatures are higher. At Lincoln University, in the dry (660 mm) coastal region of Canterbury, maximum daily soil surface temperatures peak in February at $\sim 38^{\circ}\text{C}$, with average daily soil temperatures of 22°C (Wilson *et al.*, 1995). Current estimates show mean air temperatures rising 3 to 4°C in the next 50 years (Salinger, 2003) and this would potentially decrease out of season germination of these annual species. Cultivars such as 'Bolta', with relatively low temperature onset ($<20^{\circ}\text{C}$) for high temperature dormancy (Figure 6.5), would reach their maximum germination potential later in the autumn, when temperature had decreased and available moisture was more consistent. This would decrease the annual yield potential (Chapter 5).

Subterranean clover had the highest thermal time requirements for germination (Table 6.1). Heavier seeds within a species seed lot have a competitive advantage as seedlings because they are able to utilise a greater supply of stored carbohydrate within the endosperm to promote initial leaf development (Black, 1957) (Section 7.1). However, between species, lighter seeds tend to germinate more rapidly (Murali, 1997; Norden *et al.*, 2009) and at lower temperatures compared with heavier seeds (Easton and Kliendorfer, 2008). Lighter seeded species may be adapted to environments where there is a brief period when conditions are suitable for germination (Norden *et al.*, 2009) (Section 7.1). Balansa clover and Persian clover are native to the Mediterranean region, including Iran, Turkey and Israel, where autumn rainfall can be brief and sporadic (del Pozo and Aronson, 2000; Dear, 2003). Rapid germination in this environment may allow these species to establish before the perennial species and confer a competitive advantage. However, germination prior to consistent autumn moisture can lead to false break and crop failure in winter annuals such as balansa clover (Monks *et al.*, 2008).

6.4.2 Seedling recruitment

Approximately 50% of the estimated seed set in the first year of flowering emerged after summer rains in December and this had increased to ~75% by the end of May (Figure 6.8). By the end of the second autumn, ~89% of the initial seed production had emerged, which left ~65 kg of seed/ha in the soil or more than 10 times the amount of seed initially sown. Even allowing for a further 75% softening in the third season, 16 kg of seed would still remain hard into the fourth year. Craig and Ballard (2000) in South Australia and Jansen and Ison (1996) in western New South Wales, Australia produced similar results and showed 50% seed softening at the first break of the season and ~75% of seed by the end of year 1. These results showed sown balansa clover is capable of producing a huge bank of seed which can be managed for regeneration in subsequent years. These results also showed balansa clover was able to re-establish in the fourth year, even after a failure in the third year (Chapter 3). Seed set in year 1 was able to persist through hardseededness into future growing seasons.

Because of seed wastage, including predation and fungi, the balansa clover monoculture grown in 2006 may have produced 800 kg/ha of seed. The total recovery rate of subterranean clover seeds in Australian experiments using similar methods ranged between 40 and 65% of the actual seed set (Rossiter, 1966; Taylor, 1972b). However, no direct predation of the seed was observed during the experiment and conservative estimates suggest less than 25% of balansa clover seeds are lost from the soil due to wastage including ant activity (Jansen and Ison, 1995).

Approximately 2.5% of the seed set in the establishment season remained in the soil at the beginning of the fourth season and was able to account for the presence of balansa seen in the 'MaxClover' grazing experiment (Chapter 3). In that experiment, balansa clover persisted as a component of a mixed sward for 5 years after the initial sowing from naturally regenerated seed. The recruitment pattern explains what was seen in the field in that grazing experiment.

6.4.3 Hard seed breakdown

A proportion of seed produced and shed to the ground at Lincoln University in November 2006 was soft and germinated with the first rains in autumn (Section 6.3.3). The temperature treatments imposed on 'Frontier' balansa clover seed six months after being harvested from Ashley Dene indicated the influence harvest date had on future seed softening (Figure 6.9). Seed harvested on 1 March 2007 had significantly higher germination percentage than seed harvested one month earlier on 31 January. However, the hardseed in balansa clover was not broken down completely using traditional subterranean clover-based incubator techniques with fluctuating temperatures (Quinlivan, 1961, 1966). This did not account for the ~75% seed softening per year seen in the field. In subterranean clover, the strophiole of a hard seed is made permeable to water through the continued expansion and contraction of the testa by fluctuating temperatures (Hagon, 1971). This may be because of differences in structure, chemical content or relative thickness or seed coat layers (Zeng *et al.*, 2005). The implication therefore is balansa clover either does not react to fluctuating temperatures the same way as subterranean clover or has a second dormancy which acts independently of physical seed coat impermeability. However, the chance of secondary dormancy was discounted because the seeds that did not germinate had not imbibed water at all and scarified seed from both harvest dates returned similarly high germination results at the beginning and the end of the temperature treatment experiment (Section 6.3.4).

6.4.3.1 Temperature

The failure of fluctuating temperatures alone to account for the breakdown of hardseed seen in the field implies the strophiole was protected from weakening under the imposed treatment regime. This may be because the expansion or contraction was not occurring in balansa clover seeds or the expansion and contraction did not provide sufficient action on the strophiole to break it open. The inability to simulate the breakdown of balansa clover hardseed seen in the field (Experiment 9) in incubators (Experiment 10) suggests the mechanism of breakdown is not the same as for subterranean clover (Loi *et al.*, 2005). The difference in seed size between balansa clover of 1.2 mg/seed and subterranean clover of 6.7 mg/seed may influence the physical nature of the seed coat breakdown (Zeng *et al.*, 2005).

The environment in the field in February may have been responsible for the increased breakdown in hard seed in seeds which were harvested at the beginning of February compared with those harvested at the end. The mean and mean maximum temperature at the site 10 mm below the soil surface increased from 19.9 and 27.0 °C in January 2007 to 21.5 and 29.1 °C in February 2007. The additional month of increased temperatures which seeds harvested on 1 March 2007 experienced before being subjected to the fluctuating temperatures in the incubators is the most likely explanation of the increased amount of seed softening.

However, the low absolute amount of seed softening seen in Experiment 10 was not expected. It also did not parallel what was seen in the field in Experiment 9. Taylor (1996) showed subterranean clover hardseed breakdown was higher in incubators than in the field when seeds were subjected to similar temperature fluctuations. When working with subterranean clover, four key points were identified as influencing the rate of hardseed breakdown in incubators. These are 1) the maximum temperature (Quinlivan, 1961), 2) duration of time spent at the maximum temperature (Hagon, 1971), 3) the amplitude of temperature fluctuation (Quinlivan, 1966) and 4) the speed of temperature change (Taylor, 1981). In Experiment 10, three out of the four were reasonably accounted for. However, the speed of temperature change was not representative of what occurred in the field during a summer/autumn period. The temperatures in the incubators changed from high to low and low to high relatively quickly (less than 1 hour in either direction). This may have been a confounding factor where the time spent at the interim temperatures was not representative of the warming and cooling that occurs in the field. An additional experiment (11) was run, piloting a new method of containing the seeds within a material of high thermal mass (sand) to slow the transitions between temperature extremes. In this experiment hardseed was still not broken down at any greater rate, albeit with unreplicated data and for only 14 days.

6.5 Conclusions

- Approximately 75% of the estimated 'Bolta' balansa clover seed bank emerged in the first autumn, with ~50% of the remaining seed emerging in the second autumn.
- More than 10 times the amount of seed sown at the beginning of the experiment remained in the soil at the end of autumn in the second year.
- Seed softening occurred in incubators using traditional methods of fluctuating temperatures simulating the diurnal fluctuations in the field. There was a ~20% increase in the amount of softening over the control. However, this did not account for the ~75% seed softening observed in the field. The influence and timing of a pre-conditioning phase was identified as another factor requiring investigation.

7 General Discussion

The aim of this study was to develop best management practices for balansa clover in a grazed pasture system based on an understanding of its key growth and development characteristics. This general discussion aims to draw the results from each of the individual chapters together and to discuss them in relation to balansa clover management within a grazed pasture system.

Re-establishment of balansa clover in a grazed situation was deemed to be successful, with the balansa clover contributing ~3 t DM/ha year for 5 years (Plot 1, Chapter 3). Each vegetative phenological stage in balansa occurred earlier than a range of other pasture species, requiring less thermal units to complete each stage (Table 7.1). Balansa clover was similar in its thermal time requirements for each of the development stages compared with the small seeded adventive annual suckling clover (*Trifolium dubium*) (0.33 mg/seed) (Boswell *et al.*, 2003). Both required ~25 °Cd to germinate and 330 °Cd from emergence for axillary leaf production, with a similar optimum temperature (Boswell *et al.*, 2003). Because of the similarity of balansa and suckling clover in their response to temperature, this adventive clover may act as an indicator of an ecological niche that balansa may be able to exploit.

Table 7.1 The thermal time requirements for germination, emergence, first leaf appearance and axillary leaf initiation for 'Bolta' balansa clover, 'Goulburn' subterranean clover, 'Demand' white clover and Suckling clover (*T. dubium*).

	Germination	Emergence	First leaf	Axillary leaves
	Tt ($T_{\text{base}} = 0 \text{ }^{\circ}\text{C}$) ($^{\circ}\text{Cd}$)			
Balansa clover	29	59	111 ¹	318
Subterranean clover	55	160 ²	230 ³	435 ³
White clover	49	109 ⁴	208 ⁴	532 ⁴
Suckling clover ²	23	146	220	341

Note: ¹Frontier', ²Lonati *et al.* (2009), Boswell *et al.* 2003 ³ Moot *et al.* (2003a). ⁴ Black *et al.* (2006a).

7.1 Seedling competition

Balansa clover (~1.2 mg/seed) required less thermal time for seedling establishment than subterranean clover (~6.7 mg/seed) (Table 7.1). Despite this, subterranean clover consistently contributed more dry matter than balansa clover throughout the 'MaxClover' experiment (Brown *et al.*, 2006; Mills *et al.*, 2008a). The competitive ability of balansa clover seedlings may be limited by physical size, rather than the rate of development. Seedling dry weight is dependent on cotyledon area which is in turn dependent on seed weight (Black, 1956, 1958). When comparing several cultivars of subterranean clover, Black (1958) showed heavier seeds produced heavier seedlings, independent of cultivar. Essentially, heavier seeds have heavier embryos which can provide for larger cotyledons to capture incoming solar radiation compared with lighter seeds. However, multiple niches exist in a farm ecosystem that can sustain several species with different survival strategies (Turnbull *et al.*, 2005).

Mediterranean annual species have several reproductive strategies that promote their persistence in different niches, rather than a single 'recipe' or ideotype (Rossiter, 1966; Cocks, 1994; Norman *et al.*, 2005; Turnbull *et al.*, 2005). Balansa clover should be considered a strongly ruderal species, within the Competitive, Stress avoiding, Ruderal reproductive scale (Grime, 1977; Norman *et al.*, 2005). When considering several annual clovers, Norman *et al.* (2005) described a strongly ruderal species as having a large number of relatively small seeds. Hardseeded ruderals, like balansa clover, persist by producing a large number of seeds that germinate when conditions are likely to promote successful establishment. This strategy contrasts with subterranean clover, which produces fewer relatively large seeds which in turn produce more competitive seedlings. A subterranean clover seed that germinates and fails to survive is of a higher cost to the plant/species than a smaller seeded species such as balansa clover (Norman *et al.*, 2005).

Detailed observations of species diversity and dominance have shown multiple species within a pasture system can be complimentary (Sanderson *et al.*, 2004; Scott and Pennell, 2006; Scott, 2008). One long-term experiment concluded that while a single species would become 'king' over time in each environment, contributing 40-50% of annual dry matter, other species will persist and would make substantial

contributions when environmental conditions are favourable (Scott and Pennell, 2006). A greater number of species within a pasture should lead to an increase in the amount and stability of yield by providing species to fit different climatic, topographical, temporal and nutrient niches within the same pasture (Sanderson *et al.*, 2004; Scott, 2008). These niches would include different levels of drainage, soil depth, and soil water holding capacity, aspect, slope, altitude, and grazing pressure, stock camps and nutrient returns. Established balansa clover plants have shown superior growth in waterlogged and saline conditions compared with subterranean clover (Rogers and Noble, 1991). These environmental conditions could occur within the same paddock as other, less suitable, niches, for example in water discharge areas, paddock hollows or free draining south facing slopes.

Because of the rate of development through the seedling phase, balansa clover is most successful when sown alone or with little competition from existing plants. This was evident in the growth room pot experiments (Section 4.3) and in the establishment year of the 'MaxClover' field experiment (Section 3.3.1).

Balansa clover is able to persist in the seed bank of a mixed pasture for at least five years and would be able to emerge and contribute dry matter and nitrogen when climatic and pasture conditions are suitable (Section 3.3.3). However, balansa clover required spelling during spring for seed set in the establishment year which subterranean clover did not. It also required summer grazing pressure to re-establish successfully.

In other temperate legumes, the ability to successfully establish is limited by their thermal time requirement to emergence (Moot *et al.*, 2000) or their slower rate of mainstem and axillary leaf development than companion species, e.g. Caucasian clover (*T. ambiguum*) (Black *et al.*, 2006b). This also disadvantages these species when sown in a pasture mix which can reduce the amount of light and moisture captured by the plant, particularly if sown with the rapidly establishing perennial ryegrass.

7.1.1 Balansa growth and development in an established pasture

Across most plots in the 'MaxClover' experiment (Chapter 3), the success of balansa clover re-establishment within a pasture was below the expected level, given the swift rate of development compared with subterranean clover. In the 'MaxClover' experiment, the deep soils probably assisted the aggressive cocksfoot to overwhelm the balansa clover seedlings which emerged with each autumn rain. It is suggested that competition for light and moisture was most limiting during seedling establishment (Dear and Coombes, 1992; Dear *et al.*, 2002).

Cocksfoot competes strongly for water, rapidly extending its leaf lamina to redevelop its canopy after autumn rain (Lee and Cho, 1985; Mills *et al.*, 2006). It seems likely that this post-drought response confers competitiveness to cocksfoot which may prevent balansa clover seedlings from successfully establishing. In particular, the cocksfoot canopy is likely to preferentially utilise the earliest available soil moisture while balansa seedlings are germinating and developing their first leaves. The soil dries under a cocksfoot sward because it is able to take up and use any moisture present at the time (Dear and Cocks, 1997; Mills *et al.*, 2006), reducing the availability to the annual species. The cocksfoot canopy expansion would also shade the balansa seedlings, reducing their ability to photosynthesise and establish self-sufficient plants. For example, one year old cocksfoot pastures limited the number of subterranean clover seedlings present 16 days after emerging in early March to 1% of the initial population (Dear and Cocks, 1997). This compared with 57% of seedlings surviving when sown with phalaris (*Phalaris aquatica*), a less summer active grass, in the same experiment.

Sowing balansa clover as a monoculture may, therefore, provide a way to maximise herbage and seed production, without making concessions to a grass component. Perennial species could then be sown in the following seasons, after >1 t/ha seed had been produced. Seed production may increase as management technology improves. 'Huia' white clover seed production per unit area has increased dramatically, doubling to upwards of 600 kg/ha through the use of herbicide for weed control and reducing white clover vegetative growth (Pyke *et al.*, 2004). Understanding these technologies and the role of pests, nutrients/fertiliser, top-

working and moisture as they apply to balansa clover seed production is an area for future investigation.

The presence of a summer growing perennial grass species like cocksfoot also has the ability to reduce soil moisture from rainfall events which would otherwise sustain pure swards of annual legumes at sufficient levels (Dear and Cocks, 1997). Because of this, the ability for balansa clover seedlings to compete with established cocksfoot is further reduced. If the initial moisture level is sufficient for imbibition and emergence and is subsequently reduced, the grass presence would create a false strike. The direct influence of the cocksfoot plants on reducing balansa clover survival was not investigated, but can be estimated from the false strike experience in 2006 at the 'Max Clover' experimental site. During the 'Max Clover' experiment, 23 mm of rain fell in four days from 8 March 2006. With 13 mm rainfall over the next 42 days, emerging balansa clover seedlings did not have sufficient moisture to survive. There was a failure of both balansa and subterranean clovers to contribute dry matter to the sward that year (Table 3.2 and Table 3.3). The maximum rainfall during the 42 day period was 2.5 mm, and was most likely transpired by the established cocksfoot or lost as soil evaporation.

When comparing turf-type perennial grass species with short-lived coloniser-type species (e.g. Shepherd's purse *Capsella bursa-pastoris*), Fenner (1978) provided a reasonable analogy to the roll balansa clover plays in a perennial pasture. Fenner (1978) showed clearly that the small-seeded, annual species which rapidly colonise bare soil are more susceptible to competition for light and other resources than established perennial species. Colonising species reacted poorly to low light conditions by either remaining short and failing to enter the canopy or etiolating so much that they were not able to support their leaflets. The management advice from Australia is to continuously graze balansa clover during establishment, although they do so without mention of stocking rate or likely frequency of repeated grazing (Craig and Ballard, 2000; Dear *et al.*, 2002). The implication is that balansa reacts by maintaining a compact initial growth habit which is able to tolerate grazing pressure during establishment which then minimises the impact of the associated grass. Maintaining a closely grazed sward during autumn is also likely to assist balansa clover re-establishment. While balansa clover has erect cotyledons, they are ~5 mm

above the soil surface (Plate 7.1), and Australian management advice suggests that they are not removed by animal grazing.



Plate 7.1 A 'Frontier' balansa clover seedling, showing cotyledons, spade leaf and one trifoliate leaf.

The inability to control the grass with grazing animals in the summer during the 'Max Clover' experiment highlighted one of the key areas of pasture management for re-establishment of balansa clover. Cool season annual clovers require bare ground to emerge into in autumn (Dear, 2003). Without sufficient grazing pressure in the summer, balansa clover failed to re-establish in the 'Max Clover' experiment (Section 3.4.3) and in grazed experiments in Australia (Dear *et al.*, 2002).

Competitive pressure also comes from the other annual legumes sown in a balansa clover pasture. Australian experience with sowing balansa and subterranean clover together has shown problems in achieving equilibrium (Dear and Coombes, 1992; Dear *et al.*, 2002). In a three year grazed field experiment in the wheat belt of south eastern Australia, balansa clover was unable to restrict the growth of subterranean clover beyond the first year (Dear *et al.*, 2002). The competition from the subterranean clover came very early after both species had emerged and shaded

smaller balansa clover seedlings. The authors suggest set stocking balansa clover after emergence might improve its competitiveness. The rapid leaf appearance rate of balansa clover (Chapter 4) most likely conveys a competitive advantage under constant grazing pressure when all seedlings are maintained at an even size.

7.2 Balansa clover development model

For this model, information will be taken from three 'Bolta' balansa clover monocultures that emerged on (a) 1 December 2005, (b) 16 January and (c) 27 April 2006 at Lincoln University, New Zealand. For the model, it is assumed that water is non-limiting and for all stages, $T_{opt} = 15\text{ }^{\circ}\text{C}$.

Each of the three crops produced one leaf after $111\text{ }^{\circ}\text{Cd}$ ($T_{base} = 0\text{ }^{\circ}\text{C}$) (Section 4.3.2), on the (a) 10 December, (b) 29 January and (c) 9 May (Table 7.2). The plants produce an additional mainstem leaf every $22\text{ }^{\circ}\text{Cd}$ ($T_{base} = 5\text{ }^{\circ}\text{C}$) (Section 4.3.3.2), so that the fifth mainstem leaf would appear (a) 14, (b) 15 and (c) 26 days after the spade leaf, approximately the same time as the axillary leaves would begin to appear (Section 4.3.4).

The timing of first flowers was dependent on the length and direction of change of the photoperiod at the time of emergence (Section 5.3.1.2). For plants emerging on 1 December 2006 photoperiod was 15.2 hours and increasing. This resulted in the shortest vegetative growing season, predicted to flower on 2 February 2007, 63 days after sowing ($591\text{ }^{\circ}\text{Cd}$, $T_{base} = 2.5\text{ }^{\circ}\text{C}$). The time spent growing vegetatively was longest in those plants that were sown immediately after the longest day (~21 December). In this example, plants that emerged on 16 January grew vegetatively for $1500\text{ }^{\circ}\text{Cd}$ ($T_{base} = 2.5\text{ }^{\circ}\text{C}$) until 30 August, 226 days later. For the same 16 January sown plants, the whole crop life cycle would be completed in 297 days. For plants that emerge 101 days later, on 27 April, the vegetative phase is 72 days less ($684\text{ }^{\circ}\text{Cd}$).

Table 7.2 The date and days after emergence (DAE) of each phenophase according to the calculated models for 'Bolta' balansa clover emerging on three dates at Lincoln University, New Zealand.

Emergence	(a) 1 Dec		(b) 16 Jan		(c) 27 Apr	
	Date	DAE	Date	DAE	Date	DAE
1 st leaf	10 Dec	9	29 Jan	13	9 May	12
5th MS leaf	24 Dec	23	13 Feb	28	4 Jun	38
Axillary leaves	28 Dec	27	17 Feb	32	4 Jun	38
Flowers	2 Feb	63	30 Aug	226	28 Sep	154
Peak flowers ¹	-	-	10 Oct	267	30 Oct	186
Mature seed	-	-	9 Nov	297	26 Nov	213

Note: ¹Based on estimates of field observations.

'Bolta' balansa clover flowered later than 'Frontier' when sown after the longest day (Section 5.3.1.1). Sowing 'Bolta' as soon as soil moisture allowed after the longest day would maximise the time spent growing vegetatively and increase the annual dry matter production. The potential would then be for high quality dry matter to be grazed in March/April (e.g. for hogget flushing) (Section 3.3.4). When sown in northern Victoria, Australia, Kelly and Mason (1986) showed that earlier establishment of balansa clover led to increased yield because of an increase in autumn growth. Also, balansa clover grows at its highest rate when temperatures are 10-15 °C as temperatures cool towards winter.

At Lincoln University, the mean daily air temperature drops consistently below 15 °C on about the 16 March (1961- 2007 long term for Broadfields Meteorological station, 2km North of Lincoln University). Sowing in January to maximise dry matter production may require a spring/summer fallow to ensure moisture content for establishment in the soil and/or the strategic use of irrigation. In years after the initial cultivation/establishment, this may also be partially achieved using herbicide or minimum tillage.

Because of the potential for increased annual yields because of the longer vegetative growing season, Bolta' balansa clover is recommended for climates with autumn rainfall or access to irrigation which allows for an early establishment (January - February). Spring rainfall (September - November) should also allow seed production one in five seasons, especially the establishment season. Where spring moisture is variable, two or more cultivars could be sown together to give range of flowering dates.

7.3 Successful re-establishment

7.3.1 The influence of seed production on future re-establishment

In Plot 1 of the 'MaxClover' experiment (Chapter 3), balansa clover was able to persist as a component of a pasture, contributing more than 3 t DM/ha five years after sowing (Figure 3.3). The difference between Plot 1 and the others was a function of seed production in the establishment year (>1000 kg/ha). By filling the seed bank with so many balansa clover seeds in the establishment year, seedlings were able to populate any favourable niche which became available over the next four seasons. To achieve the level of seed production which was required to maintain 'Bolta' balansa clover in the sward in the coastal region of Lincoln, Canterbury, New Zealand, the 1st year balansa based pastures needed to be closed for flowering by mid September (Section 3.3.3), just in time for the animals to be shifted on to lucerne.

The need for a large seed set in the 1st year to maintain balansa clover persistence in a sward agreed with modelling work by Jansen *et al.* (1996) in central-west New South Wales, Australia. They showed monocultures of balansa clover would survive perpetually because of their high seed production each year under their management regime where other species would not. In their dryland environment where rainfall is concentrated in winter months (524 mm/year rainfall), balansa clover seed production varied from 400 to 800 kg/ha (Jansen and Ison, 1996). This was sufficient seed to ensure the subsequent surviving seedling population of around 2000 seedlings/m² in spring the following year.

7.3.1.1 Oversowing balansa clover

The potential to improve hill country productivity through the oversowing of balansa clover is also worth considering. In their native Mediterranean environment, the soft seeds of annual legumes germinate and emerge on the soil surface in autumn following the first rains. These Mediterranean annual species are typified by adaptations which confer the ability to capitalise on available resources, including rapid emergence and a broad range of flowering times (del Pozo and Aronson, 2000). Because of this, balansa clover could be an appropriate species to include in an oversown mixture given minimum competition and suitable fertility for establishment. Balansa clover rapidly emerges (Figure 4.2) and develops leaves (Figure 4.5 and Figure 4.7) and leaf area (Figure 4.10). The ability to survive waterlogged conditions through winter and perform well in a wide range of pH (4.8-9.0) (Weir *et al.*, 1984) and produce early season dry matter (Mills *et al.*, 2008a) suggests balansa clover could make a useful contribution in developing hill and high country blocks.

The paddock to be broken in could be burned, heavily grazed and/or treated with herbicide in the year prior to sowing the legume seed to reduce competition from resident vegetation. Some herbage cover is desirable at establishment to increase humidity at the soil surface for germination of grasses (Cullen, 1966). However, because of the small seed of balansa clover and the rapid germination and emergence (Table 7.1), the need for cover to increase humidity may not be as great as the requirement for space for establishment. Initial seed mixtures should be legume dominant to increase the fertility of the block before grass species are introduced, and include a range of species to target different paddock niches (Sanderson *et al.*, 2004). Monitoring the nutrient status and suitable management techniques will help control grass weeds that may invade as the fixed nitrogen levels rise.

For this reason balansa clover seed, like most legumes being sown into virgin soil, should be inoculated (commercial Group CS (WSM409)) before oversowing to ensure rhizobia are present (Ballard *et al.*, 2002). Autumn sowing is recommended to capitalise on the vegetative production potential of balansa clover sown before the shortest day and to take advantage of available moisture. Large mobs of animals

could be used after sowing to trample seed into contact with the soil to improve emergence and establishment (White, 1990). Balansa clover performs well when grazed from emergence to control weed species (Dear *et al.*, 2002). Covers of 800-1000 kg/ha are recommended (Leigh *et al.*, 1995; Dear *et al.*, 2002). Animals should then be removed in early/mid September to allow the balansa clover to flower. Animals should be re-introduced in late summer or early autumn to graze the standing herbage and create space for balansa clover re-establishment. The grazing pressure required to control summer herbage may dictate that the paddock be subdivided, a general requirement when developing hill country.

7.4 Future work

7.4.1 Flowering and seed production

Future work should be focused on understanding more about the flowering pattern of balansa clover. The need to define 'peak flowering' for harvestable seed yields and the interaction of grazing on the timing of reproductive development would allow better management of grazing and for seed production in spring. The interaction between seed yield and spring irrigation/drought to quantify what makes a 'good' year for seed production in a grazed system should also be studied in conjunction with the likely increased development of hardseed in favourable years compared with drier seasons (Donald, 1959).

7.4.2 Potential use away from traditional dryland environments

Balansa clover has been targeted as a dryland species because it completes its annual lifecycle within the shortened growing season. However, this work has shown the potential for vegetative growth from January to October with 'Bolta' balansa clover. Work with balansa clover in higher rainfall areas, especially with winter waterlogging or saline soils (Rogers and Noble, 1991), may identify other niche growing environments. However, the slow or low breakdown of hardseededness and controlling summer herbage cover in these environments may ultimately limit the use of balansa clover. Perhaps a cultivar with softer seed, or a different pattern of seed softening would be more favourable in these situations.

7.4.3 Selection characteristics

Cultivar development has traditionally focussed on dry matter yields through winter activity and flowering date to vary the length of the growing season required (Snowball, 1994). Results from the present experiments, however, suggest that selecting balansa clover for seedling vigour and competitiveness when sown in a pasture would be the most beneficial for pastoral agriculture. The potential to select for these traits using seed size is limited, because of the low variation in the available germplasm (Snowball, 1994). However, selection for larger leaflets may provide some advantage at this early stage. A comparison with the growth form of Persian clover (Figure 4.11), as an example of a species with large leaflets, could further direct selection criteria. Additionally, the selection for a faster leaf appearance rate may be possible (Figure 4.7) and could promote seedling vigour by establishing leaf area more rapidly than currently available cultivars.

7.4.4 Additional comments

When conducting future work with Mediterranean annual legumes such as balansa and Persian clovers, it is important to be aware that they have optimum temperatures for development between 8.5 and 15°C (Section 4.3). This is ~10 °C lower than for white clover (Black *et al.*, 2006a). Because of this, the temperature ranges chosen for field and growth room experiments involving balansa clover should be different than for white clover. Work with thermal time requirements in temperate environments should therefore focus on the 5 to 15 °C range.

Further work on assisting balansa clover establishment could focus on 1) the time and severity of grazing. 2) The time and rate of herbicide application for control of the competing species. 3) The use of top-working in summer to create space for balansa clover to colonise and to conserve moisture for early balansa clover establishment. 4) Fertiliser requirements for sustained growth of balansa clover without the adverse promotion of the companion species. 5) Decreased sowing rate or row spacing of perennial grass components. 6) Using balansa clover as a one-off specialist pasture

sown as a monoculture with a pre-emergence herbicide, e.g. Trifluralin (Treflan) to control grass and broadleaf weeds, for lamb finishing.

7.5 Conclusions

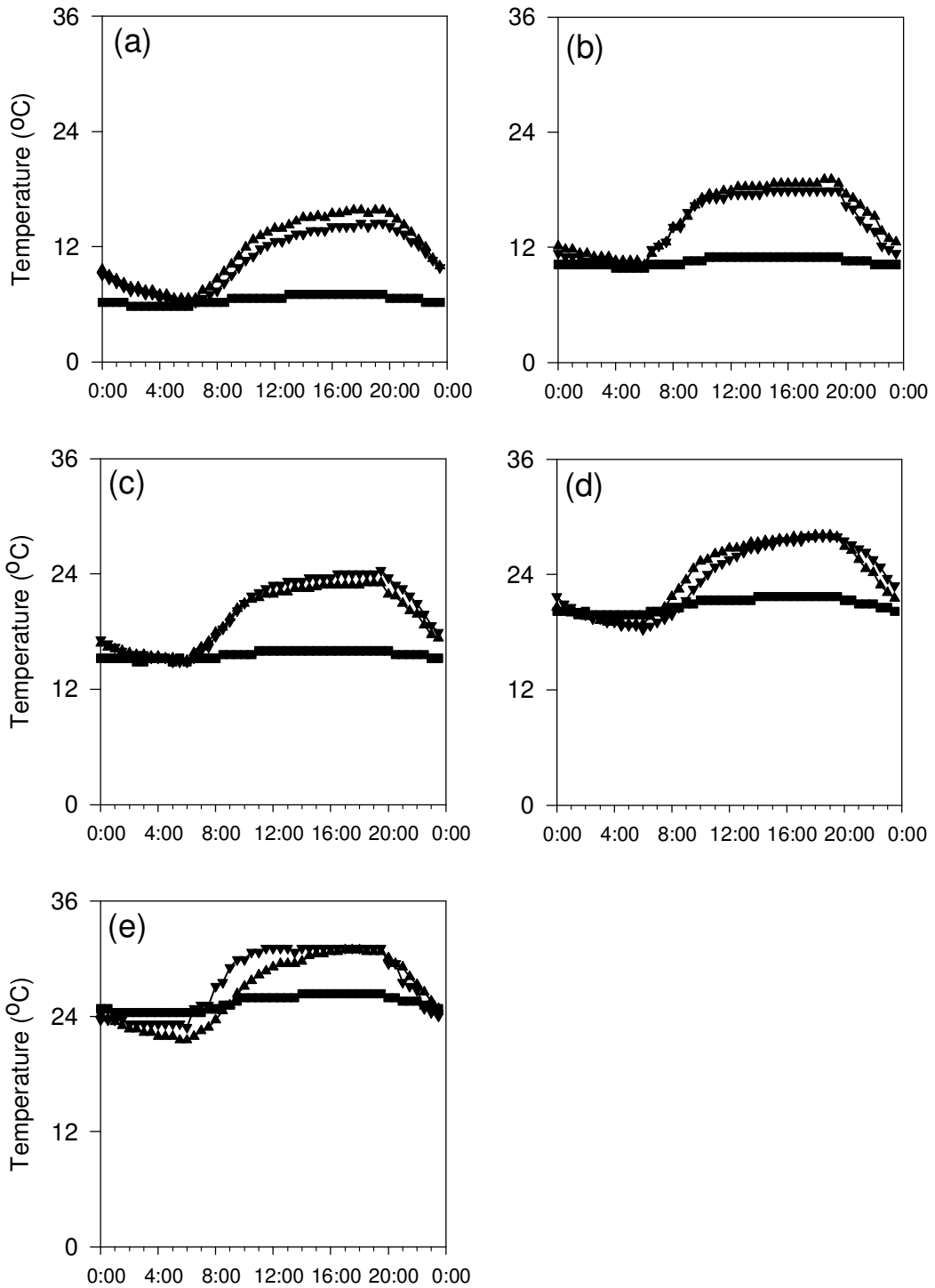
This research programme has quantified the stages of phenological development of balansa clover and discussed them in relation to farm practice. From two field-based experimental sites, growth room experiments and an incubator experiment, the following conclusions for balansa clover management can be made:

- Continued contribution to a pasture is dependent on seed production in the establishment year and the reduction of pasture cover in summer.
- 50 per cent of seeds remaining in the seed bank were recruited as seedlings each season.
- Phenological development was driven predominantly by thermal time.
 - Soil temperature explained the rate of germination and emergence. At 15 °C, balansa clover took two days to germinate and a further two days to emerge. The base temperature for germination was 0 °C.
 - Air temperature explained the rate of first leaf appearance, phyllochron and the timing of axillary leaf appearance. At 15 °C, mainstem leaves appeared every two and a half days. The base temperature was 2.5 °C.
 - Air temperature was used to define the time between pollination and physiological maturity. At 15 °C, it took 20 days and a further eight days for the seed pod to shatter. The base temperature was 2.5 °C.
- Plants which emerge into decreasing daylengths (January to July) require more thermal time to flower. This extended the period of vegetative growth beyond those which emerged after the shortest day into increasing daylengths.

8 Appendices

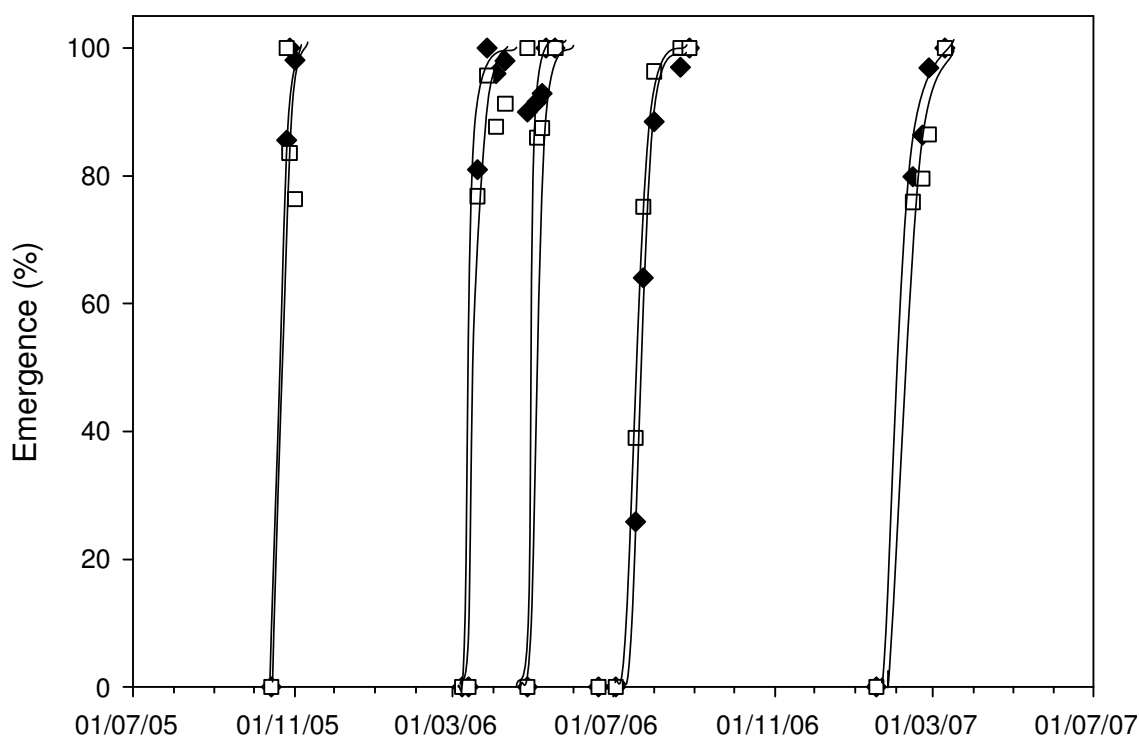
Appendix 1 The number of replicates used for statistical analysis of mean metabolisable energy (MJ/kg DM) for the legumes component of three binary pasture mixtures (sown with cocksfoot) at Lincoln University, New Zealand in the 'MaxClover' experiment.

Date	Balansa clover	Subterranean clover	White clover
21/11/2002	4	4	4
11/12/2002	4	4	4
8/01/2003	-	-	4
20/02/2003	-	-	2
21/02/2003	-	-	2
23/04/2003	-	-	4
28/05/2003	-	-	3
23/07/2003	3	2	2
24/07/2003	1	2	1
27/08/2003	4	4	4
6/10/2003	4	4	4
4/11/2003	4	4	4
11/12/2003	-	-	1
29/06/2004	5	3	4
30/08/2004	5	5	4
5/10/2004	6	6	5
2/11/2004	6	6	5
30/11/2004	6	6	5
14/12/2004	1	-	-
5/01/2005	3	1	4
1/02/2005	1	-	6
10/05/2005	3	5	-
27/06/2005	4	5	1
11/08/2005	4	5	3
20/12/2005	1	-	3
16/03/2006	-	-	1
10/11/2006	6	-	-
11/12/2006	4	6	6
8/01/2007	3	4	6
5/02/2007	-	4	6
22/03/2007	-	-	3
30/04/2007	-	-	2
26/06/2007	-	6	2
28/08/2007	2	2	1
27/09/2007	2	4	2
29/10/2007	2	4	2
27/11/2007	-	2	1



Appendix 2 The temperature recorded in Experiment 3-7 over a 24 hour period. The air temperature in each experiment was a) 6, b) 10.5, c) 15.6, d) 20.8 and e) 25.5 °C. (■) is air temperature, measured at 1 m above the growth room floor; (▼) is soil surface temperature and (▲) is the mean of two 10 mm soil temperatures.

Note: Probes recording air and soil surface temperature were covered with aluminium foil. The growth room was a Conviron PGV36 and temperatures were recorded using Thermistors KTY-110 temperature sensors and HOBO data logging equipment.



Appendix 3 The emergence of 'Bolta' (◆) and 'Frontier' (■) balansa clover sown at different times at Lincoln University, Canterbury, New Zealand. See Table 4.2 for sowing dates. Lines are generalised logistic functions from Equation 4.1.

Appendix 4 The coefficients, coefficient of determination (R^2) and standard error of the mean for logistic curves fitted to cumulative emergence data for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand.

		<i>B</i>	<i>M</i>	<i>C</i>	R^2 (%)	s.e.
'Bolta'	14/10/2005	1.62	10.87	101.20	100	1.8
	8/03/2006	1.79	11.13	108.90	100	1.8
	27/04/2006	3.80	5.88	92.90	*	*
	3/07/2006	0.27	18.57	95.79	99	3.3
	5/02/2007	0.18	20.53	98.53	99	4.8
'Frontier'	8/03/2006	0.38	6.60	119.80	69	32.3
	27/04/2006	1.33	10.86	93.66	98	5.4
	3/07/2006	0.93	4.75	96.05	97	7.1
	3/07/2006	0.28	16.56	102.89	100	2.3
	5/02/2007	0.15	20.60	95.09	96	7.6

Note: s.e. is standard error of the mean. Logistic curves are described in **Equation 4.1**.

Appendix 5 The coefficients for linear relationships between the rate of emergence at temperatures between the base and optimum and optimum and maximum for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand. Based on (a) 1200 mm above ground air temperature and (b) 10 mm soil temperature.

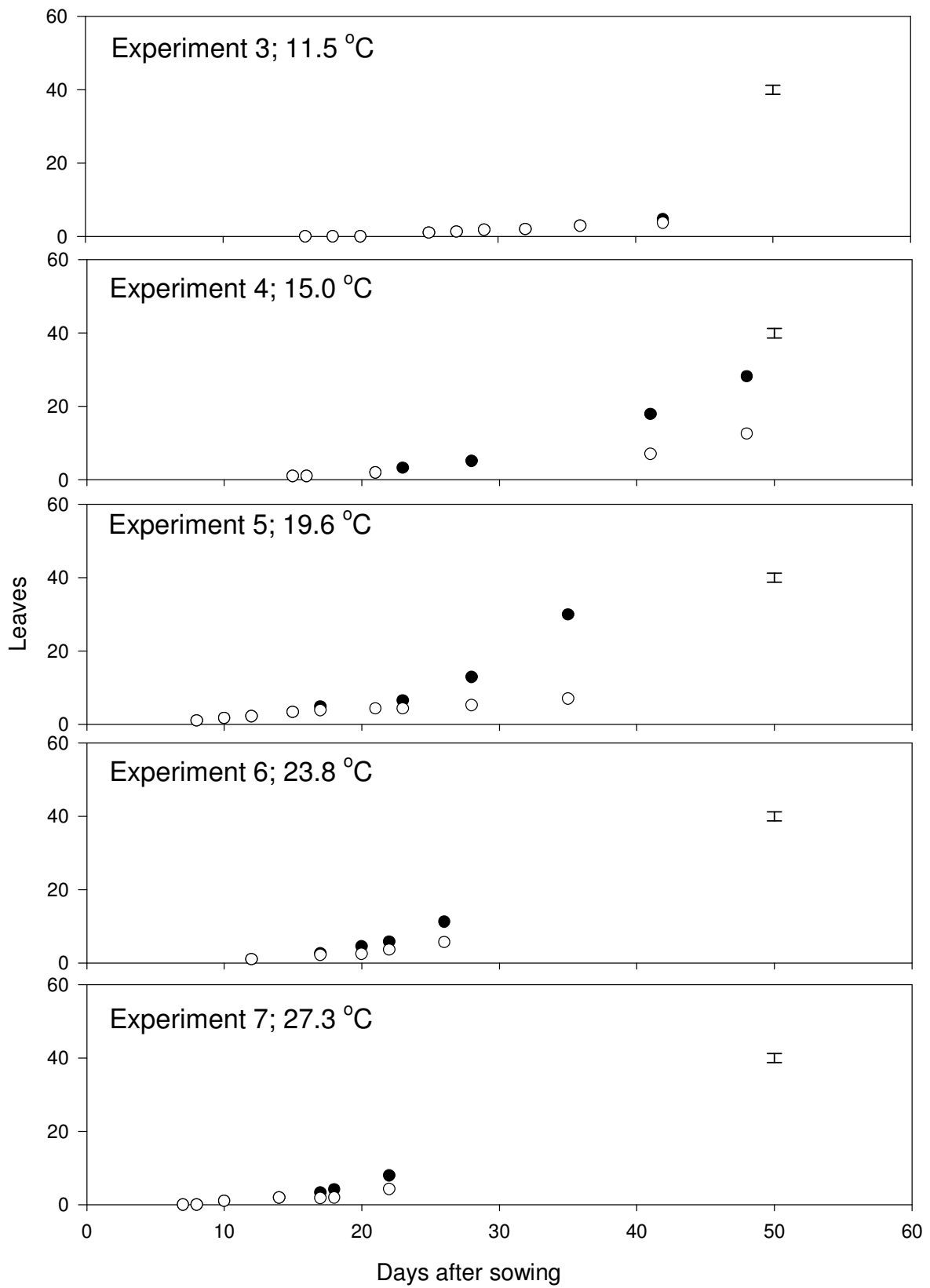
(a) Air temperature		<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Bolta'	T _{base} - T _{opt}	0.049	-0.211	-	-
	T _{opt} - T _{max}	-0.017	0.301	98	0.002
'Frontier'	T _{base} - T _{opt}	0.063	-0.282	-	-
	T _{opt} - T _{max}	-0.024	0.389	98	0.003
(b) 10 mm soil temperature					
'Bolta'	T _{base} - T _{opt}	0.026	-0.054	*	*
	T _{opt} - T _{max}	-0.014	0.287	96	0.002
'Frontier'	T _{base} - T _{opt}	0.034	-0.080	*	*
	T _{opt} - T _{max}	-0.020	0.368	95	0.004

Note: s.e. is standard error for *a*.

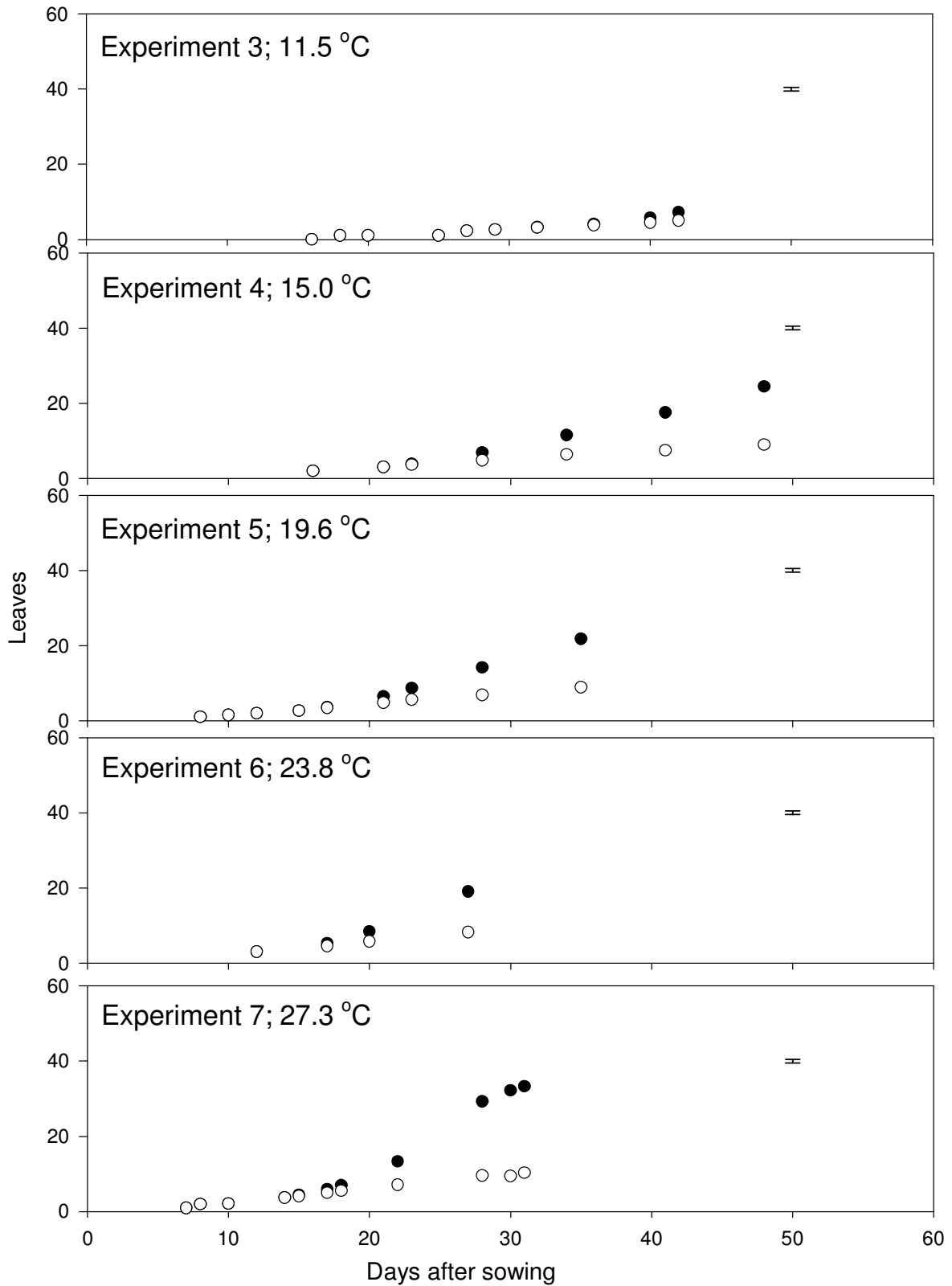
Appendix 6 The coefficients for linear relationships between the timing of the first leaf and the mean 10 mm soil temperature for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand.

	<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Aries'	0.0043	0.0020	96	0.0006
'Frontier'	0.0104	-0.0245	93	0.0021
'Laser'	0.0217	-0.1876	87	0.0047

Note: s.e. is the standard error for *a*.



Appendix 7 The number of mainstem (○) and total (●) leaves for 'Aries' perennial ryegrass sown in growth rooms.



Appendix 8 The number of mainstem (○) and total (●) leaves for 'Laser' Persian clover sown in growth rooms.

Appendix 9 The coefficients and standard error (s.e.) of the slope for linear regression analysis between the number of mainstem leaves and days after sowing for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover sown in growth rooms at Lincoln University, New Zealand.

	Mean air temperature (°C)	<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Aries'	11.5	0.16	-2.95	99	0.009
	15	0.24	-3.02	100	0.010
	19.6	0.32	-1.56	99	0.025
	23.8	0.32	-3.25	92	0.055
	27.3	0.23	-1.63	75	0.078
'Frontier'	11.5	0.24	-2.64	99	0.009
	15	0.22	-1.06	98	0.012
	19.6	0.34	-1.97	99	0.015
	23.8	0.44	-2.32	100	0.011
	27.3	0.55	-3.95	99	0.021
'Laser'	11.5	0.18	-2.56	100	0.005
	15	0.22	-1.43	100	0.007
	19.6	0.30	-1.64	100	0.007
	23.8	0.48	-3.16	90	0.114
	27.3	0.38	-1.58	99	0.011

Note: s.e. is standard error for *a*.

Appendix 10 The coefficients and standard error (s.e.) of the slope for exponential curves fitted between the total number of leaves and days after sowing for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover sown in growth rooms at Lincoln University, New Zealand.

	Mean air temperature (°C)	<i>m</i>	<i>b</i>	R ² (%)	s.e.
'Aries'	11.5	0.091	0.125	99	0.171
	15	0.111	0.220	98	0.202
	19.6	0.124	0.508	98	0.144
	23.8	0.188	0.133	100	0.120
	27.3	0.077	0.965	27	1.296
'Frontier'	11.5	0.107	0.251	98	0.161
	15	0.094	0.565	99	0.144
	19.6	0.156	0.345	98	0.196
	23.8	0.192	0.357	100	0.073
	27.3	0.073	1.600	96	0.156
'Laser'	11.5	0.072	0.296	96	0.182
	15	0.046	1.190	94	0.166
	19.6	0.124	0.477	98	0.136
	23.8	0.134	0.647	100	0.087
	27.3	0.106	0.868	62	0.584

Note: s.e. is standard error for *b*. Equations of the form $y = b.m^x$

Appendix 11 The coefficients for linear relationships between the rate of leaf appearance for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover grown in growth rooms at Lincoln University, New Zealand.

	<i>a</i>	<i>b</i>	R ²	s.e.
'Aries'	0.020	-0.061	99	0.0024
'Frontier'	0.021	-0.055	99	0.0034
'Laser'	0.024	-0.123	93	0.0046

Note: s.e. is the standard error for *a*.

Appendix 12 The coefficients for the linear relationship between the number of mainstem leaves and the number of days after sowing for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand.

		<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Bolta'	14 October	0.012	-0.23	99	0.00079
	1 December	0.012	-0.95	92	0.00241
	16 January	0.008	-2.24	96	0.00092
	8 March	0.031	-17.62	96	0.00474
	27 April	0.020	-7.42	96	0.00234
	3 July	0.009	-0.67	-	-
'Frontier'	14 October	0.008	1.60	98	0.00087
	1 December	0.010	0.70	90	0.00236
	16 January	0.006	-1.21	88	0.00133
	8 March	0.026	-14.00	98	0.00275
	27 April	0.017	-5.24	97	0.00164
	3 July	0.008	-0.15	-	-

Note: s.e. is the standard error for *a*.

Appendix 13 The coefficients for linear relationships between the phyllochron (rate of leaf appearance) for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand.

		<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Bolta'	T _{base} - T _{opt}	0.045	-0.223	98	0.0067
	T _{opt} - T _{max}	-0.069	1.146	88	0.0185
'Frontier'	T _{base} - T _{opt}	0.038	-0.187	98	0.0055
	T _{opt} - T _{max}	-0.058	0.942	69	0.0276

Note: s.e. is standard error for *a*.

Appendix 14 The coefficients for the linear and exponential regression between the rate of appearance of axillary leaves and the mean air temperature (°C) for 'Bolta' and 'Frontier' balansa clover sown in the field at different times at Lincoln University, New Zealand.

		<i>a</i>	<i>b</i>	R ² (%)	s.e.
Linear	'Bolta'	0.00220	0.00052	74	0.000917
	'Frontier'	0.00186	0.00209	89	0.000471
		<i>a</i>	<i>b</i>	<i>r</i>	s.e.
Exponential	'Bolta'	0.0135	0.0000591	1.505	0.00214
	'Frontier'	0.0134	0.0000446	1.527	0.00104

Note: s.e. is the standard error of *a*.

Appendix 15 The coefficients for the linear regression between the rate of appearance of axillary leaves and the mean 10 mm soil temperature (°C) for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover sown in growth rooms at Lincoln University, New Zealand.

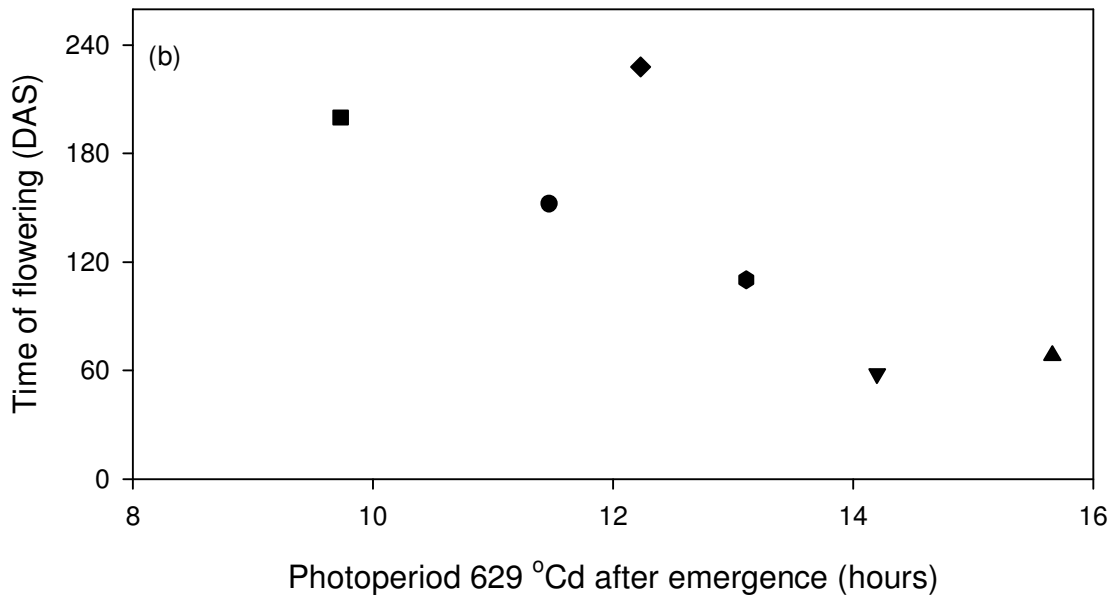
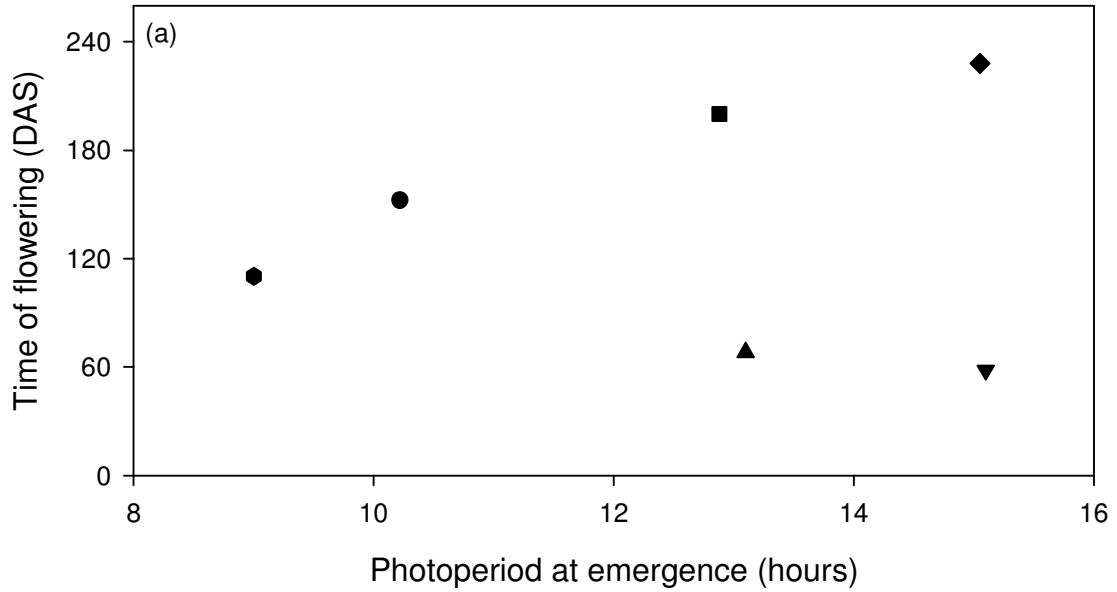
	<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Aries'	0.00528	-0.0278	100	0.000649
'Frontier'	0.00411	0.0045	100	0.000097
'Laser'	0.00423	-0.0093	94	0.001088

Note: s.e. is the standard error of *a*.

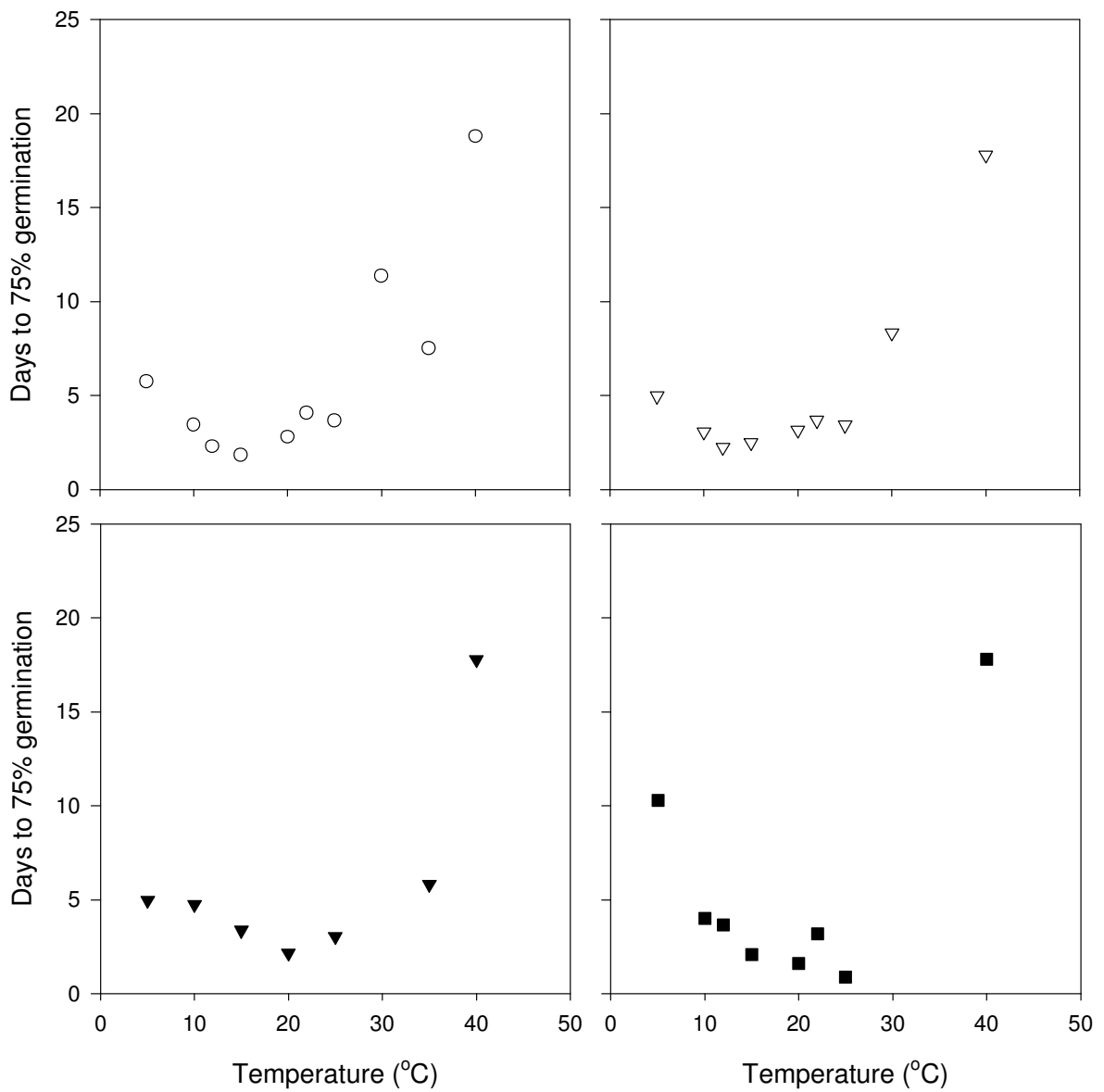
Appendix 16 The coefficients for the linear regression between leaf area and plant weight for 'Aries' perennial ryegrass, 'Frontier' balansa clover and 'Laser' Persian clover sown in growth rooms at Lincoln University, New Zealand.

	<i>a</i>	<i>b</i>	R ² (%)	s.e.
'Aries'	0.0099	-0.0769	94	0.000640
'Frontier'	0.0054	-0.0195	94	0.000367
'Laser'	0.0043	-0.0035	97	0.000189

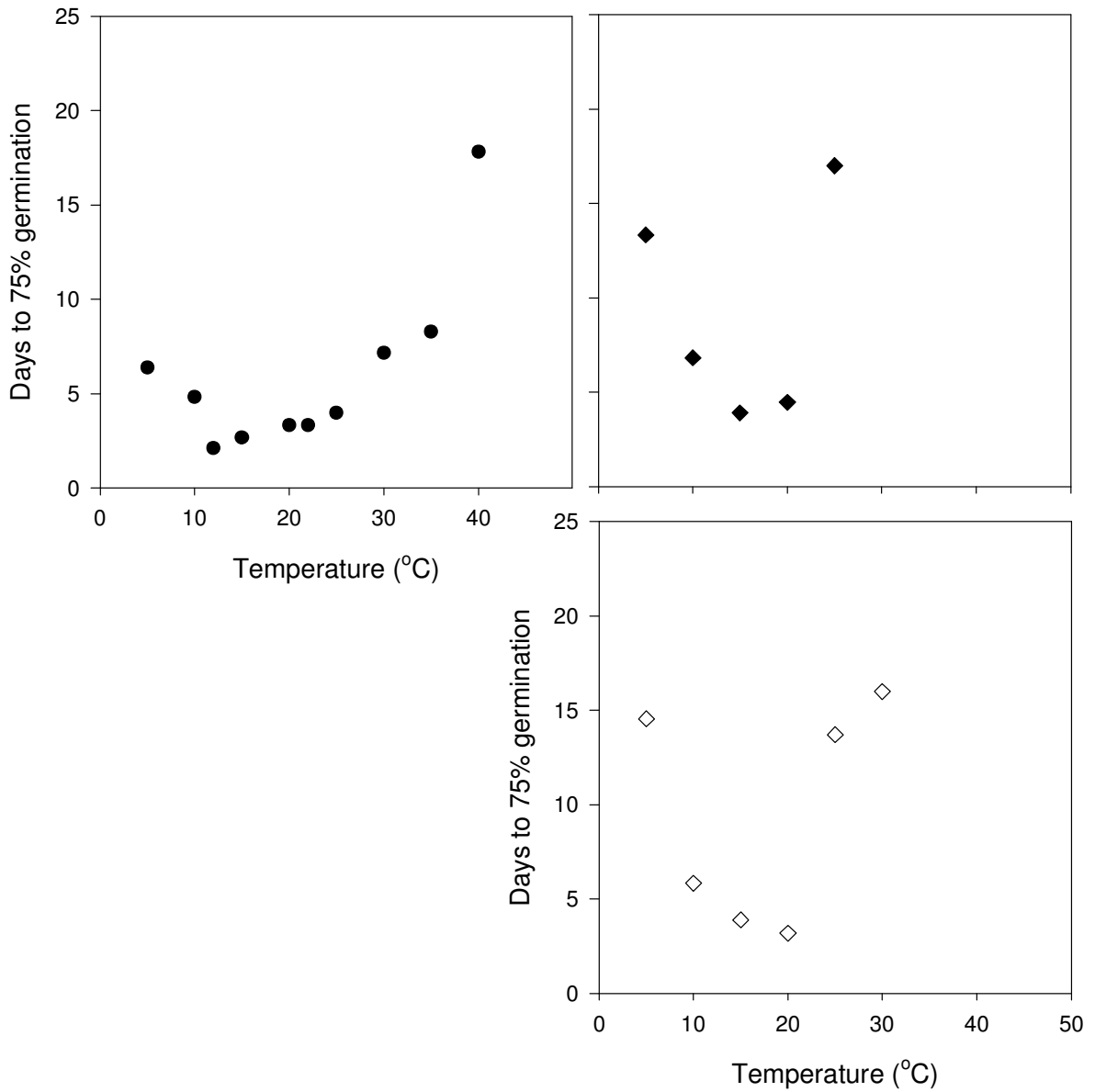
Note: s.e. is the standard error of *a*.



Appendix 17 The time of first flowers (days after sowing) for 'Bolta' balansa clover sown at Lincoln University, New Zealand on six dates against the photoperiod 629 °Cd after emergence. 629 °Cd represents the least amount of thermal time required for 'Bolta' to flower from any of the six sowing dates (Section 5.3.1.3.1). Plants were sown on 14 October (▲), 1 December (▼), 16 January (◆), 8 March (■), 27 April (●) and 3 July (⬢).



Appendix 18 Number of days to 75% of final germination for 'Bolta' (○) and 'Frontier' (▽) balansa clover, 'Demand' (▼) white clover and 'Laser' (■) Persian clover at different temperatures in incubators at Lincoln University, Canterbury, New Zealand.



Appendix 19 Number of days to 75% of final germination for ‘Nitro’ (●) Persian clover and ‘Goulburn’ (◆) and ‘Leura’ (◇) subterranean clover at different temperatures in incubators at Lincoln University, Canterbury, New Zealand.

Appendix 20 The coefficients and standard error for the linear regression between the rate of germination (DAS75^{-1}) and constant temperature below and above the optimum temperature sown in incubators at Lincoln University, New Zealand.

		<i>a</i>	<i>b</i>	R^2 (%)	s.e.
'Bolta'	$T_{\text{base}} - T_{\text{opt}}$	0.0381	-0.037	95	0.00598
	$T_{\text{opt}} - T_{\text{max}}$	-0.0180	0.724	84	0.00352
'Frontier'	$T_{\text{base}} - T_{\text{opt}}$	0.0336	0.024	95	0.00787
	$T_{\text{opt}} - T_{\text{max}}$	-0.0143	0.606	93	0.00201
'Demand'	$T_{\text{base}} - T_{\text{opt}}$	0.0204	0.030	92	0.00336
	$T_{\text{opt}} - T_{\text{max}}$	-0.0194	0.837	99	0.00138
'Laser'	$T_{\text{base}} - T_{\text{opt}}$	0.0502	-0.251	92	0.00728
	$T_{\text{opt}} - T_{\text{max}}$	-0.0726	2.962	-	-
'Nitro'	$T_{\text{base}} - T_{\text{opt}}$	0.0390	-0.070	67	0.02767
	$T_{\text{opt}} - T_{\text{max}}$	-0.0143	0.610	97	0.00109
'Goulburn'	$T_{\text{base}} - T_{\text{opt}}$	0.0181	-0.022	99	0.00217
	$T_{\text{opt}} - T_{\text{max}}$	-0.0165	0.515	90	0.00391
'Leura'	$T_{\text{base}} - T_{\text{opt}}$	0.0163	-0.002	98	0.00150
	$T_{\text{opt}} - T_{\text{max}}$	-0.0250	0.774	78	0.01322

Note: s.e. is the standard error for *a*.

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