The development and growth of annual clovers for use in dryland pasture

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By Hollena Nori

A series of field and controlled environment experiments aimed to quantify development and growth characteristics of arrowleaf, balansa, gland and Persian clovers for introduction to New Zealand dryland pastures. Thermal time requirements for germination were higher for arrowleaf (34 ^{o}Cd) than balansa (32 ^{o}Cd), gland (28 ^{o}Cd) and Persian (25 ^{o}Cd) clovers. Persian clover had an optimum germination temperature (T_{opt}) of 33 ^{o}C and a maximum temperature (T_{max}) of 45 ^{o}C, while all the other species had T_{opt} between 16-20 ^{o}C and T_{max} of ~34 ^{o}C. In field and controlled environment experiments, arrowleaf and gland clovers emerged after 90 and 96 ^{o}Cd respectively. Balansa and Persian clovers took ~89 ^{o}Cd to emerge when sown at soil temperatures below 12 ^{o}C. Above 12 ^{o}C, they emerged quicker at 75 ^{o}C d for balansa and 55 ^{o}C d for Persian clover.

Arrowleaf clover produced its first (spade) leaf after 196 ^{o}Cd compared with balansa (169 ^{o}Cd), gland (175 ^{o}Cd) and Persian (154 ^{o}Cd) clover. Throughout all sowing dates, phyllochron was the fastest for gland (33-91 ^{o}Cd/leaf) and slowest for arrowleaf (53-116 ^{o}Cd/leaf) compared with balansa (44-82 ^{o}Cd/leaf) and Persian (61-93 ^{o}Cd/leaf) clovers. Crops sown into a decreasing photoperiod had a longer phyllochron than those sown into an increasing photoperiod. The influence of photoperiod in modification of the phyllochron affected time to axillary leaf production. A slower phyllochron resulted in longer time to the first axillary leaf.

Autumn sown crops that emerged into a decreasing photoperiod had a longer thermal time requirement to flower than spring sown crops and produced more vegetative growth. Therefore, autumn sowing is recommended to maximise dry matter production. The time to flower became shorter with increasing photoperiod until the longest day of the year before it began to slow down as photoperiod decreased towards autumn. All species are long day plants, which only flower when photoperiod increases above 11.3 hours for arrowleaf, 12.6 hours for balansa, 10.3 hours for gland and 15.4 hours for Persian clover. Gland clover flowered earlier (500–1216 ^{o}Cd) compared with balansa (600-1733 ^{o}Cd), arrowleaf (940-1834 ^{o}Cd) and Persian (1047-2610 ^{o}Cd) clovers. The differences in flowering time suggests the suitability of gland clover to be grown in areas that dry out quickly in late spring, balansa clover in areas of wet winter and dry summer, and arrowleaf and Persian clovers in areas that receive high spring rainfall.

In the establishment year, these annual clovers produced dry matter up to 17.5 t/ha for balansa, 12.5 t/ha for Persian, 11.0 t/ha for gland and 9.4 t/ha for arrowleaf clover. Balansa clover had the highest radiation use efficiency of 2.1 g DM/MJ PAR absorbed followed by gland clover (1.6 g DM/MJ PAR) then arrowleaf and Persian clovers (1.3 g DM/MJ PAR). In the second year, regenerated dry matter production at full flower was up to 11.6 t/ha in balansa, 8.3 t/ha in Persian, 2.9 t/ha in gland and 0.5 t/ha in arrowleaf.

Maximum seed production of balansa and gland clovers was 2309 and 2370 kg/ha, respectively in contrast to arrowleaf and Persian clovers which only yielded 914 and 814 kg/ha seeds. In the second year, the percentage seedling emergence from the seed population was 23% for balansa, 19% for Persian, 14% for gland and 10% for arrowleaf clover. These seedlings grew to produce a ground cover up to 91% for balansa, 17% for Persian, 65% for gland and 6% for arrowleaf clover by the end of the second year. Persian clover did not persist because it did not produce any hard seeds. For arrowleaf, balansa and gland clovers, the recovery of hard seeds in the soil after 18 months showed that there was adequate seed production that could sustain on-going regeneration in subsequent years.

Key words: *Trifolium vesiculosum, T. michelianum, T. glanduliferum, T. resupinatum,* leaf appearance, growing degree days, reproductive, daylength, light interception, canopy expansion, biomass, establishment, seed softening, buried seeds.

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1 General Introduction

New Zealand is highly reliant on pastoral agriculture for its economy. This means that farming systems need to continuously produce pastures of high quality and persistence. Traditionally, the pastures are grass and legume based. Legumes are important because they fix nitrogen from the atmosphere and provide high nutritive quality forage. The most common legume which dominates most pastoral regions in New Zealand is the perennial white clover (*Trifolium repens* L.). However, white clover rarely thrives after two years in dryland environments because the taproot starts dying after about 18 months (Brock *et al.*, 2000). This causes white clover to be susceptible to drought stress especially during dry summer seasons when rainfall is lower than demand in significant areas in New Zealand (Knowles *et al.*, 2003). This limitation of white clover has led to the search for alternative legumes with greater persistence, drought survival and capable to provide early spring grazing for lambing.

Annual clovers can be used as cool season forages due to their faster growth in cool weather than perennial clovers. They have high nutritive value, with 18-25% crude protein and 60-80% dry matter digestibility) (Allinson *et al.*, 1985; Knight and Watson, 1977). Sheep tend to selectively graze clover in preference to grass and prefer a diet of 50-70% clover (Cosgrove *et al.*, 1999). Annual clovers are sown in autumn, reach their peak growth during spring, then die and set seeds in late spring/early summer. Thus, annual clovers avoid summer drought as seeds and then re-establish in the following autumn.

Subterranean clover (*Trifolium subterraneum* L.) has been grown as the dominant annual legume species in New Zealand and its role has been reviewed by Smetham (2003). In contrast, top flowering annual clovers which are the focus of this study have received less attention. Specifically, this research will focus on arrowleaf (*Trifolium vesiculosum* Savi), balansa (*Trifolium michelianum* Savi), gland (*Trifolium glanduliferum* Boiss) and Persian (*Trifolium resupinatum* L.) clovers which are commercially available in New Zealand but have not been investigated in a comparative sense. Most of the species used and cultivars sown are imported from Australia. They may increase the diversity of pasture components in mixed swards and identify species that have adaptation to a wide range of soil conditions, fast early growth, drought tolerance and can be oversown in difficult terrain. They could be used to extend grazing into summer, offer pest and disease resistance and offer species that can readily regenerate.

Arrowleaf clover is a late maturing species that can be used to extend the grazing period for lamb finishing and improve soil fertility for future cropping (Evans and Mills, 2008). Balansa clover is a mid-flowering species that can produce high dry matter and has the capacity to generate a huge seed bank that can be managed for regeneration in subsequent years (Monks *et al.*, 2008). In addition, balansa clover has been shown to persist well under waterlogged and mild salinity in Western Australia (Evans and Snowball, 1993). Gland clover is an early flowering species, pest resistant (red-legged earth mite (*Halotydeus destructor*) and aphids) and has a delayed pattern in hardseed breakdown, which prevent them from ‘false break’ (Dear *et al.*, 2001; Dear *et al.*, 2002). Persian clover is a late flowering species that has high dry matter production during spring and tolerates waterlogging (Charlton and Stewart, 2003).

At the present, there is little work presented and commercialised on the potential and performance of these annual clovers in New Zealand pasture systems. Most of the current knowledge is from Australian research and may not be directly applicable to New Zealand. Little is known about the physiological characteristics of these species which relates to their establishment and persistence in response to environmental factors. To understand these physiological characteristics, it is necessary to explore the life cycle (germination, emergence, leaf appearance, flowering, senescence and seed production) and growth (dry matter production and radiation use efficiency) of these annual clovers in response to seasonal changes in temperature and daylength. Understanding these factors is the first step to develop management strategies that might be successful under New Zealand pasture conditions.

1.1 Research objectives

The aim of this research was to understand processes related to growth and development of arrowleaf, balansa, gland and Persian clovers, when grown in a temperate climate. To achieve this aim, several experiments were carried out with the following objectives:

1. To quantify the thermal time requirements and cardinal temperatures for vegetative developmental stages i.e. germination, emergence, leaf appearance and axillary leaf production.

2. To quantify the time of first flower and duration from flowering to physiological maturity.

3. To validate phenology models derived from field experiments in Lincoln University by a series of controlled environment experiments and the field experiments off site throughout the South Island of New Zealand.

4. To quantify pasture growth in relation to dry matter production, light interception and radiation use efficiency.

5. To quantify seed production and examine the pattern of seedling recruitment and breakdown of hardseeds over time.

1.2 Proposed research structure

An outline of the thesis structure is shown in Figure 1.1. Chapter 2 is a review of literature that reports earlier research on annual clovers and factors that govern their growth and development. Chapter 3 reports on vegetative development of these annual clovers from incubation and field experiments. In Chapter 4, the time of flowering in response to photoperiod and reproductive development will be presented. Chapter 5 validates the phenology models derived in Chapters 3 and 4. Chapter 6 describes dry matter production, in response to light interception, leaf area expansion and radiation use efficiency of annual clovers. Chapter 7 reports on seedling regeneration and the breakdown of hard seed, followed by a general discussion in Chapter 8 that integrates all of these results to propose the potential role of each clover in New Zealand.

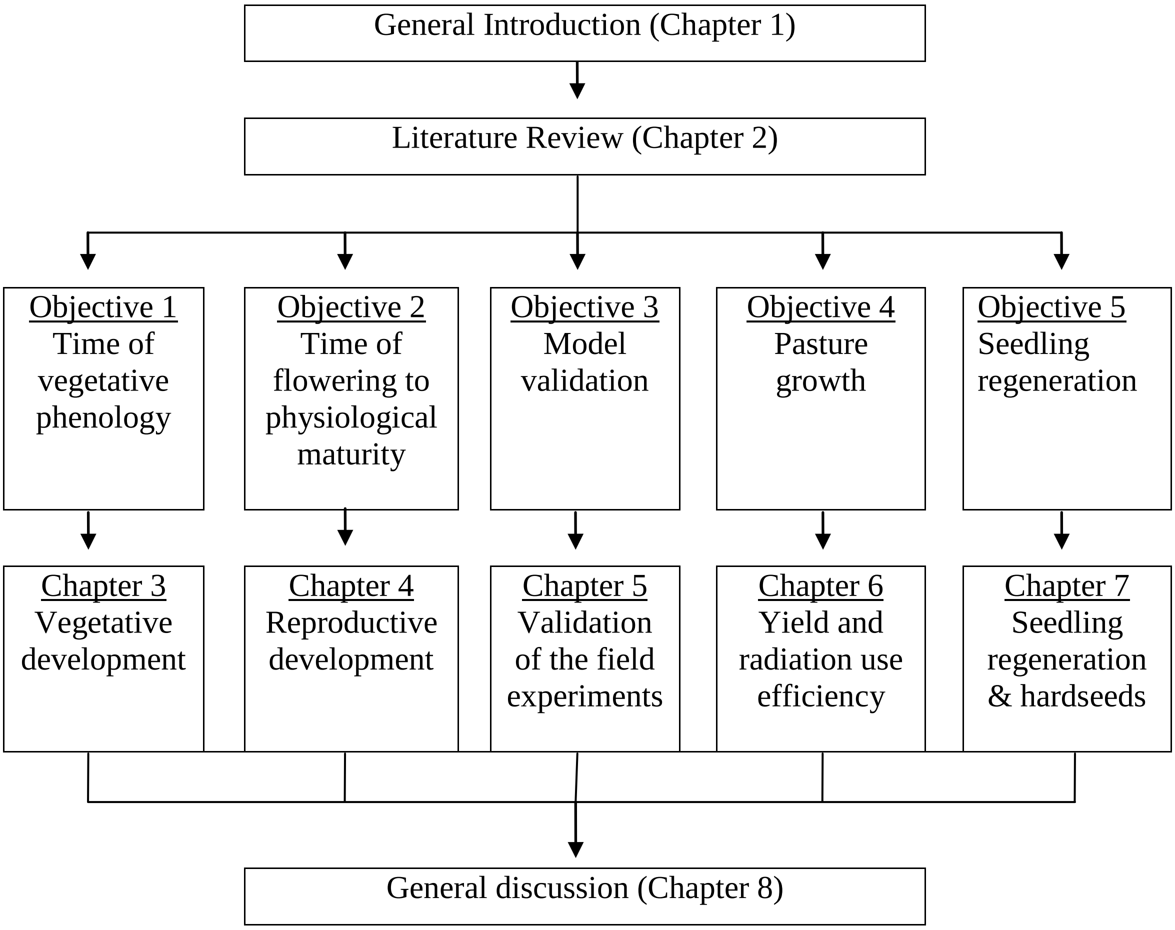


Figure 1.1 Thesis structure

2 Literature Review

2.1 Introduction

In this literature review, there is a background of New Zealand dryland pasture. This is then followed by an introduction to cool season annual clovers. The development of these legumes is described from germination and emergence through to physiological maturity. This review highlights thermal time as the main driver of plant development. The influence of photoperiod as a secondary factor in controlling the rate of leaf production and time of flowering is then discussed. Plant growth is described by the accumulation of dry matter as the result of intercepted light by the canopy. The conversion of intercepted light into biomass reflects its radiation use efficiency. Following seed production, the germination and seedling regeneration from the hardseeds of annual clovers is discussed as an important step in species persistence in dryland environments.

2.2 Background of New Zealand dryland pasture

Dryland (rainfed) farming regions make up a significant proportion of the eastern sides of the North and South Islands of New Zealand. A total of 2.87 million hectares of land (10.7% of the total land area) receives less than 800 mm of annual rainfall (Brown and Green, 2003). In these areas, pastures are prone to soil moisture deficits or drought conditions during the summer period because evaporation often exceeds rainfall. The rainfall deficit can be quantified by the potential soil moisture deficit (PSMD) and higher values of PSMD indicate higher losses of soil moisture. A PSMD level above 100 mm is considered insufficient to maintain growth of perennial pasture species, and values above 150 mm cause severe loss of pasture production (McAneney *et al.*, 1982). In the dry east coast regions of New Zealand, the PSMD increases as the summer progresses and values accumulate to between 300-500 mm, with values approximately 100-150 mm starting in December. Further deficits of 120-150 mm accumulate in January and February (Salinger, 2003). The combination of drought and high temperatures during the summer period severely limits production and persistence of white clover (Charlton and Stewart, 2003; Knowles *et al.*, 2003). This is because the white clover taproot dies after 18 months (Chapter 1) and high temperatures are the main cause of stolon death (Kendall and Stringer, 1985). Thus, white clover is not reliable in summer dry conditions.

Similarly, red clover (*Trifolium pratense* L.), which is another commonly sown perennial legume, also fails to maintain a functional taproot after the second year of production because the taproot is susceptible to pathogenic fungi and insect infestation (Kendall and Stringer, 1985). Red clover also lacks persistence as it hardly survives more than 3 or 4 years without re-establishing from reseeding. To overcome the lack of taproot longevity, Caucasian clover (*Trifolium ambiguum* M. Bieb) has been introduced to pastures because it is shown to be tolerant of several diseases (Barnett and Gibson, 1975). This species has underground rhizomes rather than above-ground stolons and has the ability to persist under dry conditions due to its deep, well developed root and rhizome system. Despite these advantages, Caucasian clover is a weak competitor in mixed swards at establishment (Black *et al.*, 2002; Moss *et al.*, 1996) which has limited its adoption as a pasture species.

Lucerne (*Medicago sativa* L.) is grown in dryland pastures because it has drought tolerance due to its deep tap root system (Scott and Sudmeyer, 1993). This means a higher PSMD can accumulate before the effects of water stress on pasture are apparent. Lucerne is capable of producing high quality forage. However, continuous use of lucerne may deplete soil moisture for following crops (Crawford and Macfarlane, 1995) and it requires rotational grazing or cutting throughout the year (Moot *et al.*, 2003b). Lucerne has slow growth during winter, and grazing must be delayed until the crop is about 0.25 m tall during early spring. This delay can result in a feed gap during the winter-spring period through gestation and lambing when a high quantity and quality of forage is needed for pregnant stock. This feed gap may be relieved by the inclusion of annual clovers in a pasture sward.

2.2.1 Hill and high country pasture

The hill and high country experience cool winters and dry summers. The temperature and rainfall are variable and influenced by altitude aspect and slope. Temperature and thermal time accumulation decrease while rainfall increases with increasing altitude. Power (2007) reported a reduction in thermal time by 100 ^{o}C d with each increment of 100 m altitude at Mount Grand, Central Otago. The north (sunny)-facing slopes are typically warmer and drier compared with south (shady)-facing slopes. The environmental variation in hill and high country resulted in the colonisation of small niches where clover species with different physiological characteristics may fit. At Mount Grand, Central Otago, dry north faces were dominated by adventive annual clovers (haresfoot (*Trifolium arvense*), suckling (*T. dubium*), cluster (*T. glomeratum*) and striated (*T. striatum*)) while white clover was reported to colonise the area of moist south faces (Maxwell *et al.*, 2010; Power, 2007). The presence of annual clovers on the north facing slopes which dry off quickly in late spring indicates their ability to complete their life cycle before the onset of summer drought. The adventive annual clover species were distributed to specific altitude and rainfall areas. Haresfoot clover occurred at the driest site at lower altitude and rainfall (Beale *et al.*, 1993; Ehrman and Cocks, 1990; Power, 2007). Mid flowering suckling clover is widespread throughout New Zealand. It covers the area from warm, moist North island hill country to high altitude tussock grassland up to 1000 m (Boswell *et al.*, 2003). According to Power (2007), suckling clover was found growing at altitude above 900 m and was the only adventive clover species found on the moist, south faces at Mount Grand. Suckling clover was also found in areas of high rainfall such as Glenfalloch, Canterbury (1665 mm annual rainfall) (Maxwell *et al.*, 2010). Late flowering cluster clover dominates the area of intermediate altitude (Power, 2007) and rainfall areas (Beale *et al.*, 1993) and grows well at the higher end of the supra-optimal temperature range of suckling clover (Boswell *et al.*, 2003). Striated clovers were present at intermediate altitude and rainfall areas (Power, 2007).

The adventive annual clovers contributed 36% of total pasture production at Mount Grand, Central Otago on the hill and high country (Maxwell, 2012). The maximum dry matter yield reported for individual species was 3.3 t/ha in haresfoot clover (Boswell *et al.*, 2003), 7.6 t/ha (with phosphorus application) and 5.1 t/ha (without phosphorus) in suckling clover (Brock, 1973) and 5.5 t/ha in cluster clover (Smith *et al.*, 1998). The importance of adventive annual clovers as nitrogen contributors to a hill and high country pastures has been recognised (Boswell *et al.*, 2003; Maxwell, 2012). However, these species are less preferred by grazing livestock (Maxwell, 2012) which may limit their spread across the entire landscape. To increase pasture productivity for feeding in hill and high country, there is a need to introduce the commercial sown annual clovers such as arrowleaf, balansa, gland and Persian clovers which are considered to be more productive and have superior feeding value and grazing preference.

2.3 Annual Clovers

Annual clovers play a role as winter dominant legumes with ability to fill in the feed gap during winter – spring period (Taylor *et al.*, 1979). They are sown or regenerate in autumn, grow rapidly before the winter (often when grasses and perennial legumes make little growth during winter), flourish in spring, mature and set seeds in early summer. The timing of spring growth and maturity are species dependent with little available comparative data. Some annual clovers that have longer maturity can extend their life span into summer. An understanding of the growth and development of annual clovers is essential for developing best management practices in a dryland pasture production system.

2.3.1 Arrowleaf Clover

Arrowleaf clover is a self-regenerating winter annual native to the Mediterranean region and southern and central Europe. In southeast U.S.A., arrowleaf clover has been grown as a forage and winter crop on more than 500 000 ha of land (Hoveland and Evers, 1995) making it the most important annual clover. Arrowleaf clover was first widely sown in Australia in the early 1980's. In Australia, arrowleaf clover has been shown to persist in areas receiving 500-600 mm of annual rainfall (Evans, 2006), which indicates that it may be suitable for dryland agricultural systems in New Zealand.

Arrowleaf clover has been shown to have slow growth and lower dry matter production during the autumn and winter than balansa, Persian and subterranean clovers (Evans, 2006). To improve winter production, arrowleaf clover can be grown in mixed pastures with annual ryegrass, perennial ryegrass, subterranean clover and annual medics. The growth of arrowleaf clover accelerates and reaches its peak during the spring and summer and eventually produces a high dry matter yield. Arrowleaf clover can grow well during summer (Evans *et al.*, 2003) because it has a deep, vigorous taproot which gives it the ability to exploit moisture stored in the subsoil. This characteristic enables arrowleaf clover to suppress growth of summer weed species. It has the potential to grow to a height of over 1.5 m which minimizes competition for light.

A field experiment conducted in Canterbury, New Zealand in 2007 showed that in the early summer, 'Arrotas' arrowleaf produced 9,800 kg DM/ha compared with 3,370 kg DM/ha from 'Leura' subterranean clover and 1790 kg DM/ha from 'Nomad' white clover (Evans and Mills, 2008). Similarly, in Victoria, Australia, 'Arrotas' arrowleaf produced over 60% of total production from late spring onwards compared with 30% for other annual legumes (Zhang *et al.*, 2004). Arrowleaf clover has 4-8 weeks longer life cycle than balansa, gland

and subterranean clovers and has the ability to utilise late spring/early summer rainfall for growth (Evans and Mills, 2008; Zhang *et al.*, 2004). This extension of the growing season in arrowleaf will also lead to the extension of the grazing period throughout summer and should result in improved livestock productivity. In addition, Holmes *et al.* (2005) reported that the extension of the growing season by 4-6 weeks in ‘Arrotas’ arrowleaf resulted in a 63% increment in lamb production compared with ‘Leura’ subterranean clover in Victoria, Australia. This indicates that arrowleaf clover has potential to be used for lamb finishing and contributing nitrogen to companion and future crops. Moreover, Peoples *et al*. (1998) quantified that arrowleaf clover with yields above 10 t DM/ha will input 250 kg N/ha into the pasture system. This is consistent with the generalised expectation that legumes fix about 25 kg N for every tonne of above dry matter produced (Peoples and Baldock, 2001).

Arrowleaf clover is adapted to well-drained sandy or clay soils with a pH range between 5-7 but it does not tolerate water logging. Due to its hard-seeded characteristics, arrowleaf clover has poor regeneration in the second season and seedling regeneration only starts increasing from the third season onwards (Zhang *et al.*, 2004) once the high level of hardseededness has broken down. Therefore, it is necessary to produce a large seed set in the first season to establish a seed bank in the soil for subsequent regeneration. To achieve this, grazing should be avoided once flowering commences in the first spring. Heavy grazing can be introduced about six weeks after flowering (when the seed heads dry off and feed nutritive value is still high). Observations suggest that a reasonable proportion (>80%) of the seed consumed by sheep will pass through the gut undamaged and still be capable of germinating (Thompson, 2005). The seedlings of arrowleaf clover are susceptible to red-legged earth mite (*Halotydeus destructor*) infestation.

There are several cultivars of arrowleaf clover with a wide maturity range. Cultivars from the U.S.A. are ‘Amclo’ (early maturing), ‘Yuchi’ (intermediate maturing) and ‘Meechee’ (late maturing). Australian cultivars are ‘Cefalu’ (early maturing), ‘Seelu’ (late maturing), ‘Zulu’ (late maturing) and ‘Arrotas’ (very late maturing). To fill a feed gap in early spring ‘Cefalu’ may be more appropriate than ‘Arrotas’ in New Zealand and will be used in this study.

2.3.2 Balansa Clover

Balansa clover, native to Mediterranean region, particularly Turkey (Craig, 1998), may complement white clover and subterranean clover in mixed pastures in New Zealand dryland regions. Balansa clover has been sown on over 1.5 M ha in Australia and was successfully grown in areas receiving 350-600 mm annual rainfall in southern and northern New South Wales (Craig and Ballard, 2000). The ability of balansa clover to thrive in low rainfall areas indicates that it has the potential to grow successfully on the dry east coast of New Zealand. Monks *et al.* (2008) grew balansa clover with cocksfoot (*Dactylis glomerata*) for six years in lowland Canterbury and showed that balansa contributed an average of 28% of the total annual dry matter. They also found that dry matter production increased significantly in the second year following heavy seed set. In addition, more than 10 times the amount of seed initially sown remained as hard seed in the soil at the end of autumn in the second year. Their findings suggest that balansa clover has the capacity to produce a large seed bank that can be managed for regeneration in subsequent years.

Balansa clover is tolerant of both rotational grazing and continuous stocking. However, grazing has to be suspended during flowering in spring to allow seed set. In established swards, light to moderate grazing can continue throughout flowering, with reasonable seed set still expected (Dear *et al.*, 2007). Monks (2009) suggested successful seed set was required once every four years to maintain balansa clover in a pasture. Balansa clover based pastures need to be grazed intensively during summer to remove herbage from the soil surface, to open the sward for seedling emergence (Craig and Ballard, 2000; Monks *et al.*, 2008) and to assist in the break-down of hard seed (Quinlivan, 1965). A high proportion of the seed consumed by livestock will pass through the rumen undigested and will be spread in dung (Edward *et al.*, 1998; Russi *et al.*, 1992a).

There are three commercially available cultivars of balansa clover that have been developed in Australia which differ mostly in flowering time. ‘Frontier’ is the earliest flowering cultivar, reaching full flower in about 100-115 days after a mid-May sowing. Being the earliest flowering it is suited to shorter growing seasons (350-500 mm annual rainfall) and will mature earlier in spring than the other cultivars. ‘Paradana’ (Craig and Beale, 1985) is of mid-season maturity (115-130 days to flower) with a mean flowering time 2-3 weeks later than ‘Frontier’ and requires 500-600 mm of annual rainfall. The latest flowering cultivar, ‘Bolta’ (Craig, 1998), flowers about 10 days later than ‘Paradana’ and is suited to higher rainfall districts (more than 600 mm) with a longer growing season. ‘Bolta’ averaged 3 t dry matter/ha/year for at least four years when sown with cocksfoot on a soil with 160-180 mm plant-available water capacity in lowland Canterbury (Monks *et al.*, 2008) and will be used in this study.

2.3.3 Gland Clover

Gland clover is a self-regenerating annual legume and originates from Golan, Israel (Nutt and Loi, 2002). It has a relatively compact growth habit, reaching a maximum height of 400-500 mm and produces small seeds, approximately 1,430,000 seeds/kg, making it similar to balansa clover (Hackney *et al.*, 2007). It tolerates waterlogged soils and is highly resistant to red-legged earth mite, blue-green aphids (*Acyrthosiphon kondoi*) and cowpea aphids (*Aphis craccivora*) (Dear *et al.*, 2001; Wang *et al.*, 1999), common pests which usually infest annual medics. In Australia, gland clover has been grown in a mixture with subterranean clover, biserrula (*Biserrula pelecinus*) and annual medics. It is adapted to a wide range of soil types ranging from neutral to mildly acidic and from well drained to mildly waterlogged (Dear *et al.*, 2001). Grazing gland clover during flowering and seed set should be spared especially in the first year of establishment to allow a large amount of seed to be set for regeneration in subsequent years. Following the establishment year, gland clover may be grazed at moderate grazing pressure through flowering and seed set without severely affecting its persistence.

Gland clover was recently introduced to areas of southern Australia with Mediterranean-type climates and low annual rainfall of 360-600 mm (Dear *et al.*, 2003a; Loi *et al.*, 2005). The first and currently only commercial cultivar, ‘Prima’ was released in 2001. It has early flowering and maturity, similar to ‘Dalkeith’ subterranean clovers. Two separate studies conducted in New South Wales, Australia on the production of gland clover grown in mixture with subterranean clover over a 3 year period found that gland clover out-yielded subterranean clover in the first year but eventually declined in productivity (less productive than subterranean clover) by the third year (Dear *et al.*, 2002). This result indicates that seedlings of gland clover that emerged from regeneration had poor competitiveness against subterranean clover. The failure of gland clover seedlings to compete with subterranean clover is related to the delayed seed-softening pattern that prevents most seeds from germinating until late in autumn. Although this characteristic is useful in resisting false breaks, it can be a disadvantage in competing against weed or pasture species that germinate rapidly with the first rains, after a summer dry period.

At present, there is no published work on the performance of gland clover in New Zealand agricultural systems but there is increasing interest in growing gland clover in dryland areas in New Zealand.

2.3.4 Persian Clover

Persian clover is native to Turkey, Afghanistan, Syria, Iraq, Iran, Greece, Portugal and Lebanon (Evans and Snowball, 1998; Lee *et al.*, 1999; Snowball and Evans, 1998). It has been commercially grown in temperate pastures of southern Australia. Persian clover is tolerant to waterlogged soils during wet-winters (Gibberd and Cocks, 1997), and has moderate tolerance to mild salinity (Evans and Snowball, 1993) compared with subterranean clover. It provides a high forage yield and has excellent regrowth potential following grazing. Persian clover has a very small seed (800,000 – 2,000,000 seeds/kg) compared with subterranean clover (150,000 seeds/kg) (Anonymous, 2003). A minimum plant population of 100 plants/m^2 is required for successful stand establishment. Hard-seeded Persian clover cultivars can be grown in a mixture with lucerne, subterranean clover, arrowleaf clover in addition to perennial and annual temperate species. Soft seeded Persian varieties can complement other annual legumes such as berseem (*Trifolium alexandrinum*), arrowleaf and balansa clovers as well as grown together with short-term ryegrass and oats (*Avena sativa*) to reduce the risk of bloat (Frame, 2005). Australian research suggests, grazing hard-seeded Persian clover throughout summer is essential to reduce herbage residues, facilitate the breakdown of hard seed and improve seedling regeneration. Due to their small seed size, the seeds of Persian clover survive digestion in the rumen of sheep and will germinate in the dung (Thomson *et al.*, 1990). Grazing in autumn is important to control weeds that can smother small Persian clover seedlings. However, grazing during late flowering and seed set is likely to be more critical as it can reduce seed production (Jansen and Ison, 1995).

There are two distinct subspecies of Persian clover namely *Trifolium resupinatum* var. *resupinatum* and *Trifolium resupinatum* var. *majus*. *Trifolium resupinatum* var. *resupinatum* has a more prostrate growth habit, produces hard-seed and has thinner stems and smaller leaflets. This subspecies is a prolific seed producer and produces a higher seed yield than var. *majus*. In addition, var. *resupinatum* flowers and matures earlier than var. *majus*. Var. *resupinatum* requires a minimum annual rainfall of 450-500 mm, which implies that it has potential to be grown in dryland pastures. There are three cultivars of this subspecies that are commonly grown in Australia namely ‘Prolific’, ‘Nitro’ and ‘Kyambro’. Both ‘Prolific’ and ‘Nitro’ are tolerant to clover scorch and *Phytophthora clandestina* while ‘Kyambro’ shows tolerance to leaf rust, stem rust and clover scorch (Anonymous, 2003). These cultivars may be candidates for use in New Zealand dryland pastures but they will not be tested in the present study.

Trifolium resupinatum var. *majus* has an erect habit, thick hollow stems and large leaflets. It is soft-seeded with late flowering and maturity traits. Late maturing Persian cultivars are suitable to sow in a mixture with subterranean clover as they can extend grazing in the late spring and early summer period when the subterranean clover has senesced (Anonymous, 2003). In Australia, cultivars of this subspecies are ‘Maral’, ‘Morbulk’, ‘Laser’ and ‘Lightning’. These cultivars are adapted to a minimum annual rainfall of 500-600 mm. ‘Laser’ is the latest flowering and is tolerant of leaf and stem rust, clover scorch and *Phytophora clandestina*. ‘Lightning’ flowers earliest and tolerates clover scorch but is susceptible to leaf rust. ‘Maral’ takes about 155-165 days to flower and it is susceptible to leaf rust. ‘Morbulk’ flowers 10 days earlier than ‘Maral’ and resists clover scorch. Cultivar ‘Mihi’, which is also from subspecies *majus*, was developed in the U.S.A. by Saddle Butte Ag Inc. ‘Mihi’ produces a high dry matter yield, large leaf size, soft seeds and is late flowering. In a commercial trial conducted on an irrigated sheep and beef farm in Canterbury, ‘Mihi’ out-yielded ‘Bolta’ balansa clover by 28%, ‘Lightning’ Persian clover by 39%, ‘Goulburn’ subterranean clover by 41% and ‘Denmark’ subterranean clover by 43% (Specialty Seeds NZ Ltd., unpublished). In the beginning, ‘Mihi’ was slower to establish than the balansa and subterranean clovers, but it managed to compete quickly throughout winter and through the summer period. Further, a glasshouse experiment showed that the root of ‘Mihi’ is resistant to moisture stress and clover root weevil (*Sitona lepidus*) infestation (Crush *et al.*, 2008). This indicates that it has potential to thrive successfully in dryland pastures of New Zealand. On the basis of these results, cultivar ‘Mihi’ will be used in this study.

2.4 Plant development

Development is defined as the chronological progression through distinct events; from germination, seedling emergence, leaf appearance, flowering and maturity within a plant life cycle (Ritchie and NeSmith, 1991). Each development event describes plant ontogenic change. For example, germination starts from imbibition of water causing the seed to swell, which is then followed by emergence and elongation of the cotyledons and the radicle. Plant development is driven by temperature and modified by photoperiod. This section will describe how temperature controls plant development rate and the influence of photoperiod in modifying the length of the development process.

2.4.1 Temperature

Temperature is an important factor that influences plant growth and development. In most cases, physiological processes and development rates increase with temperature. However, extremely high or low temperatures can be detrimental to plant processes. The range of temperatures under which growth and development respond optimally is dependent on the type of crop and species.

In the germination processes, as soon as seed dormancy is broken and the seed has imbibed, the percentage and rate of seed germination depends largely on temperature (Bewley and Black, 1994). Germination of balansa and Persian clover was shown to be inhibited at temperatures above 30^{o}C and even if seeds happened to germinate, they produced malformed seedlings (Jansen and Ison, 1994). Under non-limiting moisture conditions, the production of leaves is driven by the accumulation of daily mean temperatures (Black *et al.*, 2002). Similarly, the rate of vegetative node appearance (stem elongation) accelerates in spring (Moot *et al.*, 2001) due to increasing temperature. In white clover, high temperature (above 15^{o}C) is associated with the decreasing production of stolon branches (Kendall and Stringer, 1985; Sturite *et al.*, 2007).

2.4.1.1 Thermal time

Since temperature differs with time and seasons, a crop will not always need the same number of calendar days to reach a certain developmental stage or complete its life cycle. Therefore, phenological development in crops can be measured by using thermal time or growing degree days (^{o}C d). Thermal time is defined as heat units that can be accumulated on a daily basis from the mean daily temperature and base temperature (T_b) (Equation 2.1).

Equation 2.1 $\quad$ Thermal time (T_t) = $\sum (T_{mean} - T_b)$

Where, $T_{mean} = (T_{max} + T_{min})/2$

The base temperature is that temperature below which no further development occurs. In each development stage, the thermal time (T_t) requirement and its base temperature (T_b) can be calculated from a least squares regression (Angus *et al.*, 1981) as shown in Equation 2.2.

Equation 2.2 $\quad$ Development rate = a + bT, $\quad T_b<T<T_{opt}$

Where:
a = y-axis intercept
b = slope of the relationship
T = temperature between the base (T_b) and optimum (T_{opt}) temperature

In this model (Equation 2.2), the development rate is defined as the inverse of the time (in days) required to reach a certain percentage of development (for instance, 75% germination). This development rate increases linearly with temperature (T) between T_b and T_{opt}. Beyond T_{opt}, the rate of development decreases linearly until no development occurs at the maximum temperature (T_{max}). By using the regression coefficients a and b, the base temperature (T_b) and thermal time (Tt) requirement for each development stage can be calculated from Equation 2.3 and Equation 2.4:

Equation 2.3 $T_b = -a/b$

Equation 2.4 $Tt = 1/b$ $T<T_{opt}$

The optimum temperature (T_{opt}) is interpolated from the intersection of the two regression lines, using 'broken-stick' procedures (Draper and Smith, 1998).

However, the method of calculating daily thermal time using the average of maximum and minimum temperatures, and then subtracting the T_b (Equation 2.1) can be misleading. This method calculates daily thermal time by only using two extreme temperature points (the maximum and minimum) and does not include diurnal temperature variations. This method also excludes the relationship of plant development with cardinal temperatures (T_b, T_{opt} and T_{max}). During winter, the minimum daily temperatures are often lower than T_b and in summer, the maximum temperatures can be higher than T_{opt}. As recalled earlier, any temperature below T_b will not produce plant development and temperatures higher than T_{opt} will reduce the development rate. Thus, the implication of not including the 'broken stick' relationship will result in inaccuracy of daily thermal time calculation.

The accuracy of thermal time calculation can be improved by: (1) incorporating the 'broken-stick' relationship with three cardinal temperatures (T_b, T_{opt} and T_{max}) and (2) using daily diurnal temperature instead of mean daily temperature. Jones and Kiniry (1986) used a mathematical model (Equation 2.5) to obtain eight three-hourly temperatures which are interpolated from the daily maximum and minimum temperatures to calculate daily thermal time.

Equation 2.5 $$0.92105 + 0.114 \times I - 0.0703 \times I^2 + 0.0053 \times I^3$$

Where $I = 1$ through to 8.

Each of these values was calculated and had a base temperature subtracted before being averaged to give a single value of daily thermal time. These daily thermal time values are cumulated into a daily thermal time sum, which is used to determine the duration of each development phase. This method has been used to quantify phenology in maize (Muchow and Carberry, 1989), lucerne (Moot *et al.*, 2001), balansa clover (Monks *et al.*, 2010) and other crops and will be used to quantify the phenology of annual clovers in this present study.

Thermal time has been widely used to quantify phenology in temperate pasture species (Black *et al.*, 2006; Boswell *et al.*, 2003; Lonati *et al.*, 2009; Moot *et al.*, 2000). When plant development is described in terms of thermal time, with units of degree days (^{o}C d), a plant should always require the same amount of thermal time to reach a certain developmental stage. The thermal time approach provides a uniform measurement of plant development which can be applied over a broad range of climatic conditions. Black *et al.* (2006) quantified thermal time requirement for crown shoot initiation in Caucasian clover grown in a controlled environment chamber (1180 ^{o}C d) and applied this information to predict the number of days to reach the same phenology at four sites in New Zealand with climatic variations. The quantification of thermal time requirements for development should also recognise the change in location of the growing point (the site of temperature perception). As plants advance into different development stages, a switch between soil temperature and air temperature may be required at stem extension (Jamieson *et al.*, 1995a; Yusoff *et al.*, 2012).

The thermal time approach based on a linear relationship (Equation 2.2) has some limitations. For most plant species, the rate of plant development is linearly proportional to temperature only within a short range of temperatures. This relationship is practically exponential with a significant tail at temperatures approaching the T_b (Angus *et al.*, 1981; Bonhomme, 2000). The convenient method to calculate the T_b is via extrapolation of the linear relationship between development rate and temperature. The consequence of this method is that it excludes the exponential relationship near the physiological (actual) T_b thus, resulted in higher estimation of T_b. In reality, there is likely to be some developmental occurring below the calculated T_b (Arnold, 1959). This suggests that the extrapolated T_b is only a statistical estimation of the physiological (actual) T_b (Angus *et al.*, 1981; Bonhomme, 2000). Because of this limitation, the use of 95% confidence interval is necessary to determine whether T_b is different from 0 ^{o}C. The justification of using 0 ^{o}C as an indicator of the minimum T_b for plant development is because water freezes at this temperature, which means that enzymatic reactions usually begin above 0 ^{o}C. When 95% confidence intervals for T_b includes 0 ^{o}C, it is plausible to accept a T_b of 0 ^{o}C and to re-analyze thermal time with a T_b set at 0 ^{o}C to enable comparisons among species (Moot *et al.*, 2000). It should also be noted that the value of extrapolated T_b is dependent on the range of temperatures used within the sub-optimal ($T \leq T_{opt}$). Thus, using the widest possible temperature range will improve the accuracy in estimation of $_{Tb}$ and thermal time requirements. From this review of the thermal time approach, this linear model is considered acceptable to define development rate with temperature and therefore, will be used in this study.

2.4.2 Photoperiod

In its simplest form, photoperiod refers to the duration of light in a cycle or daylength. Photoperiod is calculated using the day number of the year and site latitude (Goodspeed, 1975). It is calculated as the duration (hours) when the geometric centre of the sun moves from 6^{o} below the eastern horizon (before sunrise) to when the sun reaches 6^{o} below the western horizon (after sunset) and therefore includes civil twilight. Photoperiod calculations include civil twilight because changes in transient light quality (mainly the ratio of red/far red light) occur at dawn and dusk (Hughes *et al.*, 1984; Smith, 1982). This is equivalent to illuminance of 3 (Griffiths, 1976) or 4 lux (Vergara and Chang, 1985), in the absence of cloud cover. These are sensed by the phytochrome, which regulates many plant development responses, hence the common term is known as 'photoperiodism'.

Plants are responsive to photoperiod for only part of their life cycle, which is between emergence and flowering. During this vegetative (pre-flowering) period, there are two phases that can be identified in plants, namely pre-inductive or juvenile and inductive phase. In the juvenile phase, the plants are only producing vegetative growth and need to reach a certain size before it can respond to the photoperiod stimulus for flowering. This means that juvenile phase is insensitive to the photoperiod and its duration is independent of photoperiod. This juvenile phase has been reported for rice (*Oryza sativa* L.) (Vergara and Chang, 1985), lentil (*Lens culinaris* Medic.) (Roberts *et al.*, 1986) and lucerne (Teixeira *et al.*, 2011) and the duration differs with species. Photoperiod only affects the duration of the inductive phase and its duration is dependent on the daylength (Roberts and Summerfield, 1987). This photoperiod induction occurs in the leaves and the flowering stimulus moves from the phloem to the meristems where flowers are to be initiated.

The response of a plant to the changes in day/night length triggers the seasonal timing for floral initiation (Garner and Allard, 1920). Photoperiodic responses of plants can be catogorized into short-day, long-day or day-neutral. Short day plants flower when the day lengths are less than a specific value. In most cases, short-day (i.e. long-night) plants flower as days grow shorter (and nights grow longer). Some examples of short day plant includes chrysanthemum (*Chrysanthemum indicum* L.), soybean (*Glycine max* (L.) Merr.) and rice. Long-day plants flower when the day lengths are longer than a specific value. These plants typically flower in spring or early summer as days are getting longer. Plants from the Mediterranean region (latitude 30–50^{o}N) are usually long day plants (Del Pozo and Aronson, 2000) and these includes clovers (*Trifolium* spp.) and lucerne. Plants from tropical regions tend to be day-neutral where flowering induction is not affected by daylength. Knowledge on the origin of plant species is important to identify whether the species possess any photoperiodic response. This is because plant species may be unable to flower when grown far from their native latitude.

Within the short and long-day photoperiodic groups, plants can exhibit a facultative (quantitative) or an obligate (qualitative) response. In a facultative response, flowering is enhanced with increasing (long-day plant) or decreasing (short-day plant) photoperiod. In contrast, obligate response required a specific photoperiod (hour) to flower.

Apart from flowering, other development aspects such as leaf appearance rate may also be affected by photoperiod. In a growth room experiment, Cao and Moss (1989) reported that increasing photoperiod accelerated leaf production in wheat, although Jamieson *et al.* (1995a) suggested this may be compromised by the site of temperature recording, and that vegetative development was predominantly controlled by temperature alone.

2.4.3 Photothermal time

Photothermal time refers to the accumulation of daily temperatures as modified by photoperiod to predict phenological development in plants. Photothermal time can be calculated from the model developed by Weir *et al*. (1984) (Equation 2.6 and Equation 2.7).

Equation 2.6 Photothermal time, $Pt = FP \times Tt$ $FP \leq 1.0$

Equation 2.7 $FP = (Pp - Pp_{base})/(Pp_{opt} - Pp_{base})$ where,

FP is the photoperiod modification factor, Pp is the photoperiod (hours) at the commencement of the photoperiod inductive phase, Pp_{base} is the base photoperiod (hours). In the absence of controlled environment data, this can be set as the shortest daylength of the year at a particular location. Equally, Pp_{opt} is the optimum photoperiod (hours) or the longest daylength of the year. In this experiment, photoperiod is calculated from sun rise to sun set, including civil twilight. For Lincoln, Canterbury, New Zealand (43^{o} 38'S, 172^{o} 28'E), the optimum photoperiod (Pp_{opt}) is set to 16.7 hours and the base photoperiod (Pp_{base}) is set to 10.0 hours to correspond to the maximum and minimum photoperiods at the site.

In contrast, Summerfield *et al.* (1991) developed a photothermal model (Equation 2.8) to predict time to flowering in annual crops.

Equation 2.8 $1/f = a + bT + cP$ where,

1/f is the rate of progress (1/days) towards flowering, a, b,and c are parameter constants specific for each cultivar, T is the mean temperature and P is the photoperiod (hour/day).

This model was used to relate the rate of progress towards flowering (1/f) to mean temperature and photoperiod from sowing to the appearance of the first flower. However, this model was based on experiments conducted in constant conditions in controlled environment growth cabinets and glasshouses. Therefore, it may not be suitable for field-based experiments where temperatures and photoperiods are fluctuating throughout the year. For this reason, the photothermal model developed by Weir *et al.*(1984) will be used in this study.

2.5 Germination and seedling emergence

Germination is a process where seeds develop the structures required to become a seedling. Germination requires imbibition of moisture and then it begins to swell. A radicle is developed and protrudes through the seed coat (testa), causing the testa to be broken. The radicle then elongates down into the soil. In clovers, germination leads to the emergence of a seedling (seminal) root and two cotyledons. These cotyledons (which contain food reserves for growth) emerge from the soil surface (epigeal) by elongation and stretching of the hypocotyl region of the stem (Figure 2.1).

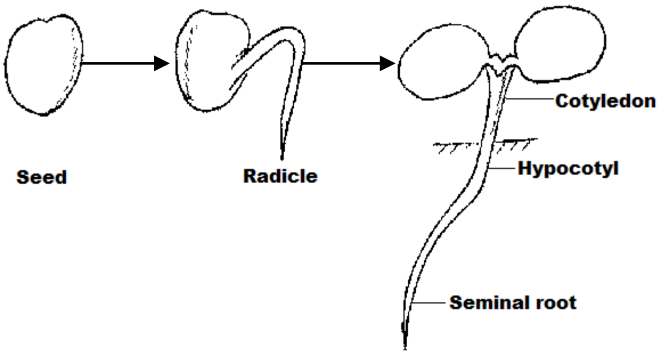


Figure 2.1 Epigeal germination of a legume seed (Adapted from Frame, (2005)).

Successful germination and seedling emergence depends on a combination of adequate soil moisture and favourable temperatures (Boswell *et al.*, 2003). Cardinal temperatures and thermal time requirements for germination and emergence have been reported for temperate legume species (Black *et al.*, 2006; Boswell *et al.*, 2003; Lonati *et al.*, 2009; Monks *et al.*, 2009; Moot *et al.*, 2000) with a T_b range from 0 to 4 ^oC, T_{opt} between 12 and 25 ^oC and T_{max} between 25 and 40 ^oC. Overall, annual species had a lower thermal time requirement for germination and emergence compared with perennial species. Specifically, Monks *et al.* (2009) reported that 'Arrotas' arrowleaf, 'Bolta' balansa and 'Mihi' Persian clovers required ~95 ^oC d to germinate with T_{opt} of 14^oC for both 'Arrotas' arrowleaf and 'Bolta' balansa clovers and 25^oC for 'Mihi' Persian clover. However, they commented that the range of temperatures used was inadequate to accurately define the cardinal temperatures and thermal time requirement for germination and gland clover was not included in their study. Thus, definition of the cardinal temperatures and thermal time requirement for germination and emergence of the four annual clover species used in this study will be investigated.

2.6 Main stem leaf appearance

Following seedling emergence, as indicated by the appearance of cotyledons, a terminal bud grows to produce a first unifoliate leaf (spade leaf) and then trifoliate leaves which appear alternately on the main stem (Figure 2.2).

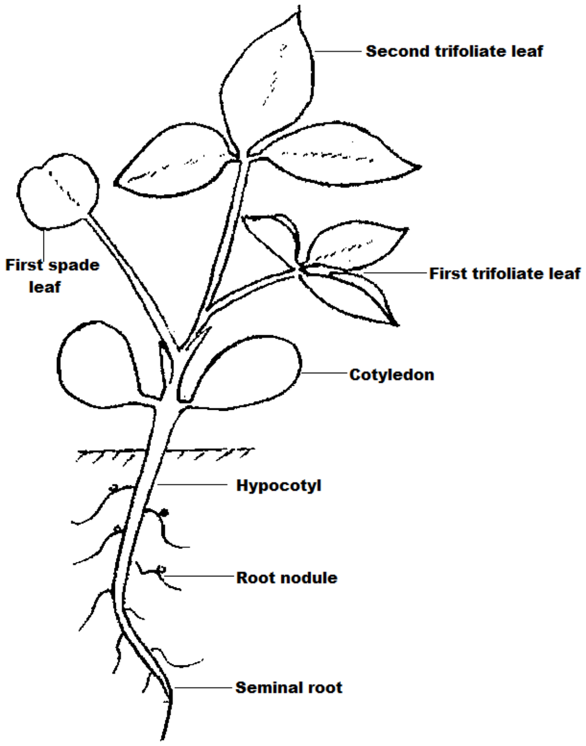


Figure 2.2 Early morphology of white clover (Adapted from Frame (2005)).

2.6.1 Phyllochron

The time interval between the appearance of successive main stem leaves is defined as the phyllochron (phyllo = leaf; chron= time) and quantified using thermal time (^{o}C d/leaf). The phyllochron is often reported to be consistent irrespective of the time of planting (Hotsonyame and Hunt, 1997; Jamieson *et al.*, 1995a; Miglietta, 1989; Slafer and Rawson, 1997) and is solely driven by the temperature at the growing point (Peacock, 1975). Jamieson *et al*. (1995a) suggested that variation of phyllochron with sowing dates was due to error in the site of temperature perception. They showed that phyllochron in wheat (*Triticum aestivum* L.) often differed across sowing dates when it was quantified using air temperature because the meristem apex was below the ground. They found that when the phyllochron was quantified using near-surface soil temperature, the result was always consistent. Thus, Jamieson *et al*. (1995a) concluded that soil temperature was the best predictor of phyllochron in wheat based on the location of the meristem apex.

In contrast, other researchers suggest that the variation of phyllochron with sowing dates was due to the effect of photoperiod at the location of seedling emergence (Baker *et al*., 1980;

Brown *et al.*, 2005; Sonego, 2000). These researchers found that phyllochron responded to the direction and changes in photoperiod length. Plants that emerged into an increasing photoperiod had a shorter phyllochron while those that emerged into a decreasing photoperiod had a longer phyllochron. A controlled environment study conducted by Cao and Moss (1989) on winter wheat and spring barley (*Hordeum vulgare* L.) also found that their phyllochron differed with photoperiod throughout the growing period. However, they did not support the hypothesis that the phyllochron is set by the photoperiod at seedling emergence. Thus one of the objectives of this study was to quantify the phyllochron of these four annual clovers in relation to temperature and photoperiod.

2.7 Axillary leaf appearance

The rate of leaf appearance determines its success in establishment and competition with other species. Specifically, the time to the first axillary leaf production is a key development phase in seedling establishment. It indicates the point in time when the plant begins to increase its leaf area exponentially and starts to form a leaf canopy where it can compete strongly for light. Therefore, plants that reach axillary leaf production earlier will have an advantage to capture more incoming radiation through the expansion of the canopy. In short, the success or failure of a species can depend on the time taken to reach axillary leaf production. For example, the extended time taken for Caucasian clover to produce its axillary leaf (after 1180 ^{o}C d) compared with 440 ^{o}C d in white clover and 373 ^{o}C d in perennial ryegrass makes it a poor competitor in a sward and therefore limits its adoption as a pasture species (Black *et al.*, 2006). In contrast, annual species produce axillary leaves much quicker compared with perennial. Monks (2009) showed that ‘Bolta’ and ‘Frontier’ balansa clovers had a thermal time requirement of 115 and 144 ^{o}C d, respectively for axillary leaf production. Validating these results for ‘Bolta’ balansa clover and quantifying the time of axillary leaf appearance for the other three species will be a part of the current experiment.

2.8 Reproductive development

The reproductive phase begins with flower initiation, when the shoot at the main stem changes from producing new young leaves to producing a flower bud. These flower buds will form inflorescences and each of these will develop into floral organs. In indeterminate species, the flowers are initiated at the axillary buds rather than the terminal bud, and the leaf appearance does not stop at the appearance of the first flowers (Burton, 1997; Sinclair, 1984). The plant continues to grow in stem length, set flowers and pods as long as temperature and moisture permit. In contrast, determinate species convert their terminal bud from a vegetative to reproductive state, resulting in the production of a terminal flower. Hence, the appearance of first flowers marks the completion of leaf canopy expansion (vegetative growth) (Burton, 1997).

The time of flower initiation is mostly determined by the final number of leaves on the main stem (Brooking *et al.*, 1995; Dracup and Kirby, 1996b; Miglietta, 1991). The number of leaves that develop on the main shoot apex before switching into reproductive development is strongly influenced by photoperiod, hence sowing date. During photoperiod induction, the leaf detects the changes between increasing and decreasing photoperiod, which enables the plant to set the number of leaves to flowering (Baker *et al.*, 1980; Hay and Delecolle, 1989). By doing this, the plant will be able to survive in a harsh environment and will only flower when conditions are favourable. For example, plants that emerge into a declining photoperiod in autumn will delay flowering by producing more leaves than those that emerge into the lengthening photoperiod in spring. This will avoid the autumn sown plants from being killed by winter frost because they will flower only slightly earlier than the spring sown crops when temperature and photoperiod increase (Brooking *et al.*, 1995).

Thus, photoperiod affects the time to flowering by adjusting the amount and duration of vegetative growth, depending on the time of sowing. Monks (2009) quantified time to flowering of ‘Bolta’ balansa clover sown on six dates between 16 January and 1 December using calendar days, thermal time and photothermal time. He found that the number of days to flowering decreased with each successive sowing date from 228 days for January sown to 58 days for December sown crops. When thermal time to flowering was quantified in relation to photoperiod, it formed a hysteresis with increasing and decreasing photoperiod at emergence. For plants that emerged into a decreasing photoperiod, the thermal time requirement to flowering decreased from 1500 ^{o}C d at a photoperiod of 15 hours (16 January) down to 630 ^{o}C d at a 9 hour photoperiod (3 July). However, the thermal time requirement to flower remained relatively constant (~620 ^{o}C d) for seedlings that emerged following 3 July into an increasing photoperiod. Quantification of flowering time used photothermal time but a single relationship could not explain the huge gap in flowering time for plants that emerged at the same photoperiod but experienced changes in opposite directions. This indicates the plants were able to perceive changes in the direction of daylength apart from being able to measure the length of photoperiod (Thomas and Vince-Prue, 1997). However, the limited number of sowing dates did not allow him to determine the optimum photoperiod to flower. In addition, time to flowering in arrowleaf, gland and Persian clovers has not been quantified. Thus, reproductive development of these four annual clovers will be quantified in this research.

2.8.1 Visual reproductive development scale

As the flower bud develops, the peduncle beneath the flower bud starts to elongate and the inflorescence starts unfolding from the base of the bud (Plate 1a) and eventually forms a corolla. The unfolding of the floret indicates that it is ready to enter into the next phase, pollination. The most basal floret of the inflorescence is the oldest and therefore the first to be pollinated. In many clover species, the floret turns brown and droops after it has been pollinated (Plate 1b). Following pollination, the ovary grows into a pod and the florets fall off (Plate 1c). The pod grows in size, changes colour and dries out as it approaches maturity. Simultaneously, the seeds within the pod continue to grow, increase in weight and change colour as they reach physiological maturity. The seed is defined as physiologically mature when the dry weight of the inflorescence is at its maximum (Hyde, 1950). Balansa clover took ~250^{o}Cd (Tb = 2.5^{o}C) from pollination to seed maturity (Monks, 2009) and this process is driven solely by temperature accumulation.

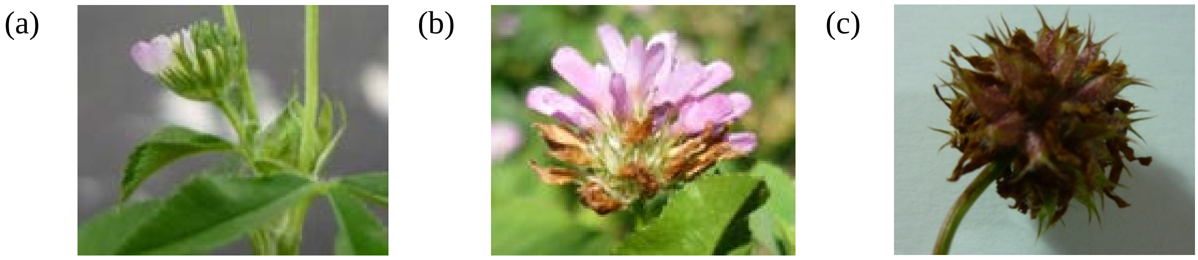


Plate 1 (a) Unfolding of the inflorescence at the base of the bud, (b) lowermost florets turned brown after being pollinated and (c) pod visible and florets fall off in ‘Mihi’ Persian clover.

A visual reproductive development scale is useful to farmers, researchers and agronomists to track physical changes as inflorescence progresses from bud visible to seed maturity. This development scale is numbered in the order of ontogenetical stages of appearance. Each ontogenetical stage is identified with a field observable picture and brief description. The use of defined and illustrated scale will be a field guide to identify the key stages of development under field conditions. This will enable farmers to decide the suitable time of seed harvesting and grazing.

The Zadoks cereal code (Zadoks *et al.*, 1974) documented developmental stages from germination to seed ripening in grain crops. This development scale has been used extensively in wheat, barley, oats, rye (*Secale cereal*) and rice. Based on Zadoks code, development scale for legume crops was produced for soybean (Fehr *et al.*, 1971) , faba bean (Knott, 1990), pea (Knott, 1987) and lupin (Dracup and Kirby, 1996a) . Later on, Monks (2009) generated a reproductive development scale for balansa clover which described the progress of individual inflorescence from bud visible to seed shatter over three seasons (2005-2007). This scale will be used to track reproductive development of balansa clover and to generate a similar scale for arrowleaf, gland and Persian clovers in this study.

2.9 Seed production

The number of seeds produced in a pod depends on the species of legume. Balansa clover produces about 3 seeds/pod, arrowleaf and gland clover have 2-3 seeds/pod while Persian clover has one seed per pod (Frame, 2005). Individual seed weight of these four species ranges from 1.1-1.7 mg/seed for arrowleaf (Wiley *et al.*, 1993) , 0.6-0.9 mg/seed for balansa, 0.7-0.8 mg/seed for gland and 0.8-0.9 mg/seed for Persian clovers (Dear *et al.*, 2003b). These small seeded species produce large populations of seeds compared with larger seeded species such as subterranean clover (Table 2.1). From an ecological perspective, this is a survival strategy. Small seeded (lighter seeds) species have smaller cotyledons which in turn produce smaller seedlings. Therefore, they have to produce a large number of seeds to secure high plant populations in a sward to be competitive. In contrast, heavier seeds have larger cotyledons and produce heavier seedlings. Thus, species with heavier seeds rely on few seeds which produce larger and more competitive seedlings.

Table 2.1 Seed weight and average seed production of balansa, gland, Persian and subterranean clovers grown in three locations at southern New South Wales, Australia in 1999. Source: (Dear *et al.*, 2003b)

Species	Seed weight (mg/seed)	Seeds/m^2	Seed yield (kg/ha)
Balansa	0.6-0.9	45000	314
Gland	0.7-0.8	39000	314
Persian	0.8-0.9	56000	495
Subterranean	4.9-7.7	3000	184

2.9.1 Hardseededness

Hardseededness refers to a seed that does not imbibe water due to impermeability of the seed coat. The process of seed coat impermeability develops during the final stages of seed ripening when seed begins to dry (Aitken, 1939; Hyde, 1954). The seed coat enters into a semi-impermeable state once the seed dries to below 14% moisture (Quinlivan, 1971). Further seed dehydration below critical seed moisture content will result in an irreversibly impermeable seed coat (Quinlivan, 1971; Standifer *et al.*, 1989). The critical seed moisture content reported in most legume species was around 7% (Argel and Paton, 1999). Once the seed has dehydrated to below the critical moisture content, it will not imbibe moisture even if placed in moist conditions. At this state of seed impermeability, the strophiole inhibits the entry of water into the seeds (Ballard, 1973; Pritchard *et al.*, 1988). Hardseededness prevents a ‘false break’, the situation where seeds germinate as a result of the first rain during late summer but then die off before sufficient rainfall for seedling survival. Hardseededness lengthens the lifespan of viable seed and distributes germination across several seasons, thus provides persistence of the species (Norman *et al.*, 2005; Norman *et al.*, 1998; Quinlivan, 1971; Russi *et al.*, 1992b). Therefore, annual clovers rely on high production of hard seeds to build up a large seed bank for their survival (Boswell *et al.*, 2003; Jansen and Ison, 1996; Kendall and Stringer, 1985).

2.9.2 Seedling regeneration

The survival of an annual species depends on the production of large numbers of seeds and the ability of the seeds to germinate and produce reasonable seedling populations in the following season. Work by Dear *et al.* (2002) and Zhang *et al.* (2004) showed that the subsequent regeneration year for arrowleaf, balansa, gland and Persian clovers produced 8, 35, 24 and 15 times the initial seedling population/m^2 in the establishment year (Table 2.2).

Table 2.2 Legume seedling density (plants/m^2) in the establishment year and total seedlings present in the subsequent regeneration year.

Species	Sowing rate (kg/ha)	Number of plants/m^2		Reference
		Establishment	1st year regeneration	
Arrowleaf	10	108	855	(Zhang *et al.*, 2004)
Balansa	6	64	2250	(Dear *et al.*, 2002)
Gland	6	104	2475	(Dear *et al.*, 2002)
Persian	10	505	7610	(Zhang *et al.*, 2004)

Following seed production, a higher proportion of seedlings regenerated in summer and autumn compared with winter and spring (Jansen, 1991; Jansen and Ison, 1996; Taylor and Ewing, 1992). Two reasons that could possibly favour regeneration of these seedlings are (1) warm temperatures in summer which induce seed softening and (2) sufficient moisture in the autumn that promotes seed germination. Jansen and Ison (1996) reported that about 65% of seedlings of ‘Paradana’ balansa clover and ‘SA12240’ and ‘SA14433’ Persian clovers regenerated over summer while 30% regenerated during autumn. Similarly, half of the seeds of subterranean clover germinated in the summer with declining proportions thereafter (Taylor and Ewing, 1992). However, this germination of seeds during summer, when rainfall is often inconsistent could lead to seedling death (‘false break’).

2.9.2.1 ‘False break’

‘False break’ refers to a condition where seeds imbibe moisture following rainfall during the summer period and germinate but eventually die due to subsequent drought conditions. This is because during the summer period, rainfall maybe inconsistent so seeds germinate and produce seedlings that are unable to survive moisture stress. In selecting legume species for pasture establishment, species with late dormancy or hardseed breakdown reduce the risk of ‘false break’ (Chapman and Asseng, 2001). Gland clover has a delayed hardseed breakdown pattern compared with balansa and subterranean clovers which prevents most seeds from germinating until the end of autumn (Dear *et al.*, 2002). While this could be an advantage in resisting a ‘false break’, it could also be a disadvantage in competition with species that establish early following first rains (Section 2.3.3).

2.9.3 Hardseed breakdown

Very high level of hardseededness often results in poor establishment in the second year (Lodge *et al.*, 1990; Smetham and Ying, 1991). Theoretically, impermeability of the seed coat can be broken by temperature fluctuations which weaken the strophiolar region and increase its permeability to water (Hagon and Ballard, 1970; Taylor, 1981). Thus, many controlled environment studies use fluctuating temperatures between 15-80 ^{o}C in an attempt to break the impermeability of hard legume seeds (Norman *et al.*, 2006; Quinlivan, 1961, 1966; Taylor, 1981; Taylor, 1993). Specifically, a fluctuating temperature of 60/15 ^{o}C has been shown to induce rapid seed softening of subterranean clover and has been adopted by the Australian National Sub Clover Program as a standard procedure to quantify hardseeds levels (Norman *et al.*, 2006; Taylor, 1981; Taylor and Ewing, 1992).

Practically, under field conditions, hardseed breakdown can be achieved by heavy grazing during summer following seed set. The removal of topgrowth opens the sward and increases daily soil surface temperature fluctuations (Quinlivan, 1965). Such an environment will increase the rate of hardseeds softening. Jansen and Ison (1995) reported that summer grazing of balansa and Persian clovers did not affect their seed bank in the autumn. Small -seeded species consumed by grazing animals are more likely to pass through the animal gut unharmed compared with larger seeds (Carter, 1980). About 23% of 2 mg (Russi *et al*., 1992) and 45% of 1 mg (Edward *et al*., 1998) seeds passed through the animal passage and returned to the soil. Similarly, Carter *et al*. (unpublished) reported that small seeds of ‘Bolta’ balansa clover (<1 mg) remained viable after passing through the sheep gut compared with larger seeds of lupin and subterranean clover (>2 mg). Quantifying hardseed breakdown in stored and buried conditions is one of the objectives of this study.

2.9.4 Buried seeds

Naturally, when seeds are dropped from the plant or passed through the animal gut, they lie on the soil surface and some are covered by the soil or animal faeces. During land preparation for the next crop, the majority of these seeds would be expected to be buried at various depths. Most of the seed populations germinate within the first year of burial (Lewis, 1961). The germination level starts to decline thereafter probably due to limited soil aeration resulted by soil compaction (Barton, 1962; Lewis, 1961). The density of buried seeds differs with burial depth (James and Rahman, 2003; Loi *et al.*, 1999; Pe, 1978). Hardseeds that are buried near the soil surface are more likely to germinate and emerge whereas those that are buried deeper in the soil, have a reduced chance to germinate. Hence, seed populations that lie near the soil surface are subjected to greater loss via germination while those buried in the soil will retain their viability much longer. It is believed that seeds buried near the soil surface had a higher germination rate due to warmer and higher amplitudes of temperature fluctuation to induce breakdown of hardseeds rapidly (Pe, 1978). At deeper burial, failure of seeds to germinate may be due to unfavourable conditions such as low oxygen supply (Harris, 1959), high carbon dioxide levels (Wesson and Wareing, 1969) and lower and relatively smaller temperature fluctuations (Darlington and Steinbauer, 1961) in breaking seeds impermeability. Therefore, hardseeds that are buried deeper will be preserved longer and able to maintain the seed population for many years. This present study will investigate the survival of buried seeds and those that lie on a soil surface over a period of 1.5 years.

2.10 Plant growth

Growth refers to the accumulation of dry matter as the result of light interception and partitioning of assimilates from photosynthetic activity. This leads to an increment of plant height, dry matter weight and leaf area expansion (Christian, 1977; Ritchie and NeSmith, 1991).

2.10.1 Light interception

The growth of plants involves energy conversion where incident light is transformed into biomass through the process of photosynthesis. During photosynthesis, incident light energy between 400 to 700 nm wavelengths is photosynthetically active radiation (PAR). This PAR is assumed as half of the incoming solar radiation (Monteith, 1972). Providing that nutrients and water are not limiting, weeds and insects are controlled and crops are grown under optimal temperature, the maximum yield of a crop is largely dependent on the availability and amount of light intercepted as PAR by the leaf canopy (Cooper, 1970; Loomis *et al.*, 1971; Monteith, 1972). This intercepted PAR is either absorbed or reflected by the leaves. The size of leaf area in a crop canopy determines how much PAR is captured by the leaf and consequently influences canopy photosynthesis and crop yield (Kendall and Stringer, 1985). The canopy of a growing crop at a given time is determined by the date of crop emergence, phyllochron, leaf expansion rate and duration, rate of axillary leaf production and rate of leaf senescence, where each of these factors can be under genetic, environmental or agronomic control (Hay and Walker, 1989).

To maximize photosynthesis, a canopy of leaves requires a leaf area index (LAI) higher than 1.0. This is because a canopy with a LAI of 1.0 will capture less than 50% of incident PAR due to large gaps in the canopy while the remaining light will strike on the ground surface and be lost from the photosynthetic system (Moot *et al.*, 2007). The LAI at which 95% of incident PAR is intercepted is defined as the critical LAI (LAI_{crit}). The value of LAI_{crit} differs among crop types and species. Species that have erect leaves will have more light penetration through the canopy, and are therefore able to intercept more light. Thus, they have a higher LAI_{crit} compared with species with horizontal leaves. A LAI_{crit} between 3-5 has been reported for potato (*Solanum tuberosum* L.) (Khurana and McLaren, 1982), wheat (Hipps *et al.*, 1983), soybean (Wells, 1991) and white clover (Brougham, 1958) while LAI_{crit} between 6-7 was reported for maize (Gallo and Daughtry, 1986), perennial ryegrass and timothy (*Phleum pratense* L.) (Brougham, 1958). The differences in LAI_{crit} can be attributed to leaf arrangement and leaf angle in the canopy which determines how they intercept the incoming PAR. The intercepted PAR by the crop canopy is distributed among the leaves at different height and arrangement. This means, some leaves (especially those at the top) are exposed to full sunlight while some (below the canopy) are illuminated by transmitted and reflected light. The transmission of light down through the canopy is quantified by the canopy extinction coefficient (k) and is influenced by the leaf orientation and angle (Hay and Walker, 1989). Canopies of erect leaves have a low k value which range from 0.2, such as barley. In contrast, horizontal leaves for species like white clover have a high k value of 0.9-1.0 (Brown and Blaser, 1968).

2.10.2 Dry matter accumulation and radiation use efficiency

The growth of plants at a given time can be described by a sigmoid curve. During the early stage of growth, dry matter production is proportional to the size of leaf area. When crops approach maturity, the accumulation of dry matter is slow because the leaf area decreases due to senescence and the efficiency of photosynthesis is reduced (Monteith 1965). Thus, the accumulation of dry matter is proportional to the intercepted PAR (Kiniry *et al.*, 1989; Sinclair and Muchow, 1999) at least until crop maturity. The efficiency of this intercepted

PAR to transform into biomass is known as radiation use efficiency (RUE). This RUE is assumed to be constant among crop species when grown under optimum conditions.

Under optimum growth conditions, intercepted PAR is converted to biomass at a rate of about 2.5 g DM/MJ PAR for temperate C_3 species (most New Zealand pasture species) and up to 3.8 g DM/MJ PAR for tropical C_4 species such as maize (Moot *et al.*, 2007). This represents an efficiency of about 4-7% of PAR captured on the assumption that 1 g of carbohydrate plant dry matter yields 17.5 kJ of energy combustion (Monteith, 1977).

There has been a wide range of methodologies used to estimate RUE. Most RUE values are determined based on intercepted PAR, others are estimated on absorbed PAR (Daughtry *et al.*, 1992; Goyne *et al.*, 1993; Green, 1987). When using absorbed PAR, it is common to assume that absorbed PAR is 85% of the intercepted PAR (Sinclair and Muchow, 1999). Most RUE estimates are also based on above ground biomass while there are some expressed on a total above and below ground biomass (Green, 1987; Hall *et al.*, 1995). Consequently, these result in variations among RUE calculations. RUE differs among species due to differences in photosynthesis efficiency. C_4 species have higher RUE compared with C_3 species (Sinclair and Muchow, 1999). The efficiency of a crop to convert captured PAR into biomass is determined by the rate of photosynthesis. Therefore, variation in photosynthesis rate will alter the crop RUE. The three main factors that influence photosynthesis activity are nitrogen, moisture and temperature. Various studies had reported on the sensitivity of RUE to leaf nitrogen content (Hammer and Wright, 1994; Sands, 1996), drought (Inthapan and Fukai, 1988; Jamieson *et al.*, 1995b; Muchow, 1985) and temperature (Andrade *et al.*, 1993; Sinclair *et al.*, 1994). To date, there are no reported values for k, LAI_{crit} or RUE of annual clover species.

2.11 Conclusion

The dryland pastures in New Zealand require legumes to provide nitrogen, improve summer feed quality and improve year round quality feed. The limitation of white clover to persist with summer drought has been recognized and lucerne has been introduced into the dryland pastures to overcome this problem. There are other legumes that have potential to be introduced into the pastoral system, particularly annual legumes which could provide short-term high quality forage but little information is known about their management practices. To date, subterranean clover remains the dominant annual legume species that is grown in New Zealand dryland farming regions, but problems with seed contamination, difficulty to oversow and susceptibility to frost heave means there may also be a place for top flowering annual clovers. To introduce these annual clovers into the pastoral system, the first step is to understand the influence of environmental factors on their growth and development and their relationship to competition and persistence in the ecological niche. Therefore, the following profile of research is proposed for the four annual clovers of interest:

1. Quantification of vegetative development: germination, seedling emergence and leaf appearance as quantified by thermal time.
2. Quantification of reproductive development: timing of flowering in response to photoperiod and duration from flowering to seed production.
3. Comparison of growth rates: dry matter production, light interception, canopy expansion and radiation use efficiency.
4. Estimation of seed yield: seed set and regeneration potential from shed seed.

3 Germination and vegetative development

Part of this chapter has been published:

Nori, H., Black, A.D., Moot, D.J. 2012. Vegetative development of four annual clovers. *In*: I. Yunusa (ed.). Proceedings of the 16th Australian Agronomy Conference, 14-18 October 2012, University of New England, Armidale, NSW. http://www.regional.org.au/au/asa/2012/crop-development/7944_norih.htm

3.1 Introduction

The development process of plant is driven by the accumulation of thermal time (Section 2.4.1.1). Developmental characteristics in seedlings determine their success in establishment and competition among species in a pasture sward. Species that have rapid germination, emergence, leaf production and canopy expansion have an advantage in competition for limited resources, particularly light. Canopy expansion is determined by the rate of leaf appearance (phyllochron) on the main stem, the time of axillary leaf production, and the subsequent expansion of leaf area.

This chapter aims to (1) determine cardinal temperatures and thermal time requirement for germination of Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers at constant temperatures in an incubator and (2) quantify thermal time requirements for emergence, first leaf appearance, phyllochron and axillary leaf appearance of these four species sown on 10 dates in Iversen 9 field, Lincoln University, New Zealand.

3.2 Materials and methods

3.2.1 Incubation Experiment

3.2.1.1 Germination

Three replicates of 50 seeds of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers were placed on wetted blotting paper in sealed plastic containers and germinated in unlit incubators at constant temperatures of 5, 8,10,12,15, 18, 20, 25 or 30^{o}C. Additional temperatures of 22.5^{o}C, 35^{o}C, 37.5^{o}C and 40^{o}C were added later, to increase the number of data points below and beyond the optimum temperature to assist determination of

T_b and T_{max}. Distilled water was added as required to ensure moisture was non-limiting for germination. Germinated seeds were counted and removed twice daily during periods of rapid germination and daily at other times to aid inspection of the remaining seeds until germination ceased (ISTA, 2004). Seeds were considered germinated when the radical exceeded the small diameter of the seed. A Gompertz model was fitted to the cumulative percentage germination against days:

Equation 3.1 $$\mathrm{CG} = \mathrm{C} \times \mathrm{e}^{\left(-\mathrm{e}^{(-\mathrm{B}\times(\mathrm{t}-\mathrm{M}))}\right)}$$

Where *CG* is the cumulative percentage of seeds germinated at time, '*t*' (days), *C* is the final germination percentage and *B* and *M* are constants. The number of days to 75% of the final germination percentage (t_{75}) was calculated using Equation 3.2 derived from the Gompertz model where *CG* = 75:

Equation 3.2 $$\mathrm{t}_{75} = \mathrm{M} - \ln[-\ln(\frac{\mathrm{y}}{100})]/\mathrm{B}$$

3.2.2 Field Experiments

3.2.2.1 Site description

3.2.2.1.1 Location

The major field experiment was located in Block 9 of Iversen Field (Iversen 9) at Lincoln University, Canterbury, New Zealand (43^{o} 38'S, 172^{o} 28'E, 11 m a.m.s.l.). Iversen 9 contained rape (*Brassica napus* L.), oats and lucerne experiments in the previous seasons, for the past 10 years.

3.2.2.1.2 Soil

The soil is classified as a Wakanui silt loam (*Udic Ustochrept*, USDA Soil taxonomy) with 1.8-3.5 m of fine textured material overlying gravels (Cox, 1978). Wakanui soils are imperfectly drained and display strong mottling below 0.7 m which indicates periods of water logging (Watt and Brugham, 1992). A soil test on 15th December 2009 showed adequate fertility for legume growth, except for sulfur (Table 3.1). On 27th January 2010, Maxi Sulfur Super (5% P, 50% S) was applied at 400 kg/ha, as maintenance fertilizer.

Table 3.1 Soil test (0-75 mm) results for the Iversen 9 block at Lincoln University, Canterbury, New Zealand on 15th December 2009.

Analysis	Result	Recommended range
pH	6.1	5.8 - 6.2
Olsen Phosphorus (µg/mL)	26	20 - 30
Calcium (me/100g)	6.7	3.0 -9.0
Magnesium (me/100g)	0.92	1.0 – 1.5
Potassium (me/100g)	0.94	0.30 – 0.50
Sodium (me/100g)	0.16	0.20 -0.40
C.E.C (me/100g)	13	12 - 25
Sulphate Sulfur (µg/g)	4	10 - 12

3.2.2.1.3 Meteorological conditions

3.2.2.1.3.1 Rainfall and evapotranspiration

Annual rainfall from 1st July to 30th June was 612 mm in 2009/2010, 602 mm in 2010/2011 and 581 mm in 2011/2012. These were below the long term mean (LTM, 1975 – 2008) of 630 mm/year (Table 3.2). Monthly rainfall ranged from 153 mm in May 2010 to 9 mm in November 2009 (Figure 3.1). Annual evapotranspiration (PET) was 1033 mm in 2009/2010, 1063 mm in 2010/2011 and 906 mm in 2011/2012, with two years above and one year below the LTM of 1024 mm/year. Monthly PET followed a similar pattern in each season, increasing from ~30 mm in July to a maximum of between 140 – 157 mm in December before decreasing to a minimum of ~20 mm in June.

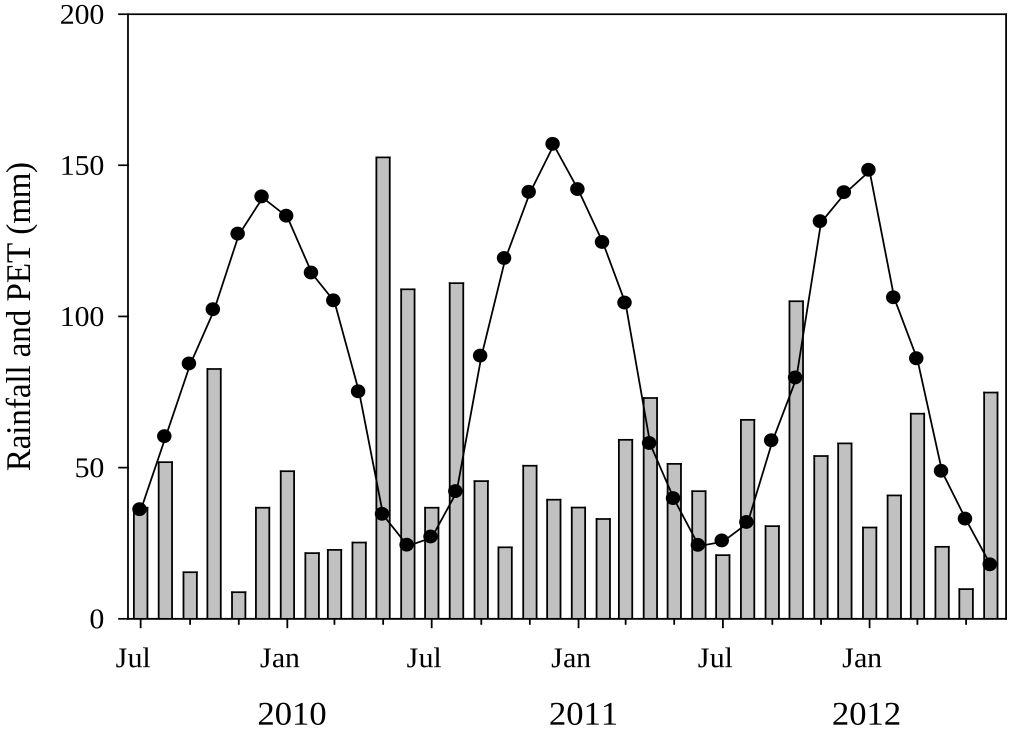


Figure 3.1 Monthly rainfall (bars) and Penman potential evapo-transpiration (PET, ●) from 1 July 2009 to 30 June 2012. Data were obtained from Broadfields meteorological station (2 km north of the site), Canterbury, New Zealand.

Table 3.2 Long term means (LTM) from 1975 to 2008 for monthly rainfall and evapotranspiration (PET) and daily solar radiation and air temperatures at Lincoln University, Canterbury, New Zealand.

	Rainfall (mm)	PET (mm)	Solar radiation (MJ/m^2/d)	Air temperature (^{o}C)
July	64	34	5.0	6.1
August	65	49	7.6	7.4
September	42	71	11.9	9.3
October	51	104	16.9	11.3
November	51	125	21.2	13.0
December	55	142	22.4	15.0
January	47	149	22.1	16.6
February	44	114	18.5	16.0
March	52	99	14.3	14.8
April	48	63	9.7	12.0
May	52	43	5.9	9.3
June	60	31	4.2	6.5
Total	630	1024	-	-

Data were obtained from Broadfields meteorological station (2 km north of the site), Canterbury, New Zealand.

3.2.2.1.3.2 Temperature and solar radiation

The mean daily air temperature followed a similar pattern in each season, ranging from 6–8 °C in June – August to 16–20 °C in February (Figure 3.2). The mean daily 100 mm soil temperature ranged from 5–8 °C in June – August to 18–19 °C in February. The mean daily total solar radiation followed a similar pattern each season, increasing from a minimum of 4–6 MJ/m^2/d in June/July to a maximum of ~23 MJ/m^2/d in December.

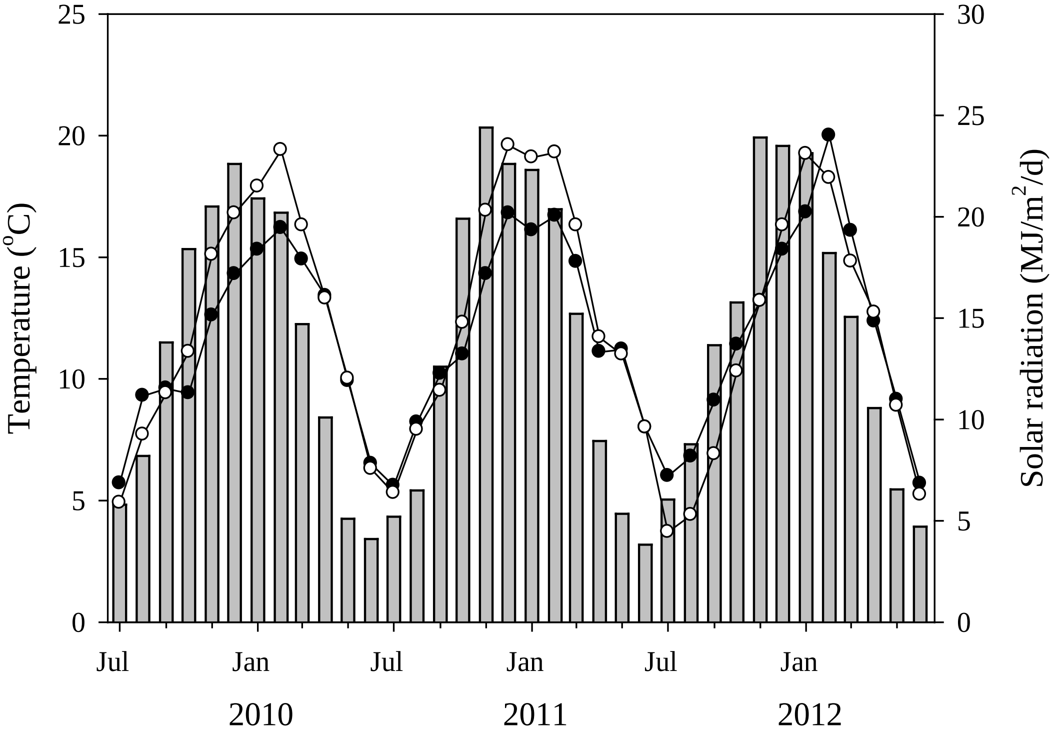


Figure 3.2 Mean daily air (●) and 100 mm soil (○) temperatures, and mean daily solar radiation (bars) from 1 July 2009 to 30 June 2012. Data were obtained from Broadfields meteorological station (2 km north of the site), Canterbury, New Zealand.

3.2.2.2 Experimental design

A split-plot design experiment with four replicates was initiated on 10 sowing dates (Table 3.3). Main plots were sowing dates, while subplots were the four annual clover species ('Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian) which gave a total of 160 plots. Each subplot measured 2 x 3 m, giving an area of 6 m^2.

Table 3.3 Sowing dates (SD) used to quantify phenological development of four annual clover species sown at Iversen 9, Lincoln University, Canterbury, New Zealand.

SD 1	26 February 2010
SD 2	30 March 2010
SD 3	4 May 2010
SD 4	3 June 2010
SD 5	7 July 2010
SD 6	14 August 2010
SD 7	25 September 2010
SD 8	9 November 2010
SD 9	20 December 2010
SD 10	19 January 2011

3.2.2.3 Sowing method

Prior to the first sowing, the experimental area was cultivated using conventional methods to produce a firm, fine seedbed. Irrigation water was applied using a rotational irrigator on 1st and 17th February 2010 with 40 mm for each date. Based on final germination percentage and seed weight data, bare seeds of clovers were hand broadcast as pure stands at 6 kg/ha for 'Cefalu' arrowleaf, 4 kg/ha for 'Bolta' balansa and 'Prima' gland and 5 kg/ha for 'Mihi' Persian, to target seedling emergence at ~400 seedlings/m^2. All seeds were sown with Group C granule inoculants (sourced: ALOSCA Tech. Pty. Ltd., Australia). The aim was to produce a monoculture of each species that could be used to monitor plant development.

3.2.2.4 Management

Before each subsequent sowing date, the unsown plots were sprayed with Roundup 360 (a.i. 360g/L glyphosate) at 3 L/ha to remove weeds. The plots were then rotary-hoed to regain a fine seed bed. All plots were irrigated with a garden sprinkler with at least 6 mm, as measured by a rain gauge after each sowing to aid establishment. Irrigation was withdrawn at first leaf appearance. Following establishment, weeds were controlled by hand as necessary.

3.2.2.5 Measurements

3.2.2.5.1 Soil and air temperature

Prior to sowing, six temperature sensors (Thermistors KTY- 110) were placed at the experimental site. Five were placed within the soil at a depth at 10 mm and one at 1200 mm above the soil surface, protected by an aluminium shield. Temperatures were logged every hour with a HOBO data logger (Onset Computer Corporation) to define the daily maximum and minimum temperatures for thermal time calculations.

Thermal time was calculated daily as the mean of 8x3 hourly temperatures generated from the maximum and minimum temperature using a sinusoidal model to account for the temperature change through a day (Equation 2.5, Section 2.4.1.1).

This thermal time calculation used a T_b of 0^oC based on the highest coefficient of determination (R^2) for least squares regression of temperatures against the rate of development.

3.2.2.5.2 Photoperiod

Daily photoperiod was determined from the latitude and longitude coordinates (Section 2.4.2). For Lincoln, Canterbury, New Zealand (43^o 38’S, 172^o 28’E), the maximum and minimum photoperiod of the year was set to 16.7 hours and 10.0 hours, respectively.

3.2.2.5.3 Emergence and first leaf appearance

After sowing, three quadrats measuring 100 x 100 mm each were placed in a fixed position on each subplot. The number of seedlings which emerged in the area was counted every alternate day until seedlings ceased to emerge. Emergence was considered to have occurred when both cotyledons were visible. The number of days after sowing to reach 50% of final emergence (t_{50}) was determined from the Gompertz model where y = 50:

Equation 3.3 $$t_{50} = M - \ln[-\ln(\frac{y}{100})]/B$$

The Gompertz model was fitted using Genstat 12.2 Statistical Software (Lawes Agricultural Trust). The number of days to reach 50% of final first leaf (spade leaf) appearance on the subplot plants was also determined using Equation 3.3. Thermal time requirements to 50% of final emergence and first leaf appearance were analysed using both soil and air temperature. Soil temperature (10 mm) was used based on a higher coefficient of determination (R^2) from least squares regression of mean temperature against the development rate. At this stage of phenology, the apical meristem, which controls growth activity and is also the site which responds to temperature, is located at the base of the plant, under the ground but close to the soil surface.

3.2.2.5.4 Leaf appearance

The number of emerged leaves on the main stem and axillary buds was counted at 4-7 day intervals on 10 marked plants per subplot until they had produced 25 or more leaves. Leaves were considered emerged as soon as the petiole was visible (Carlson, 1966). The interval of each successive leaf on the main stem (phyllochron (^{o}Cd/leaf)) was calculated from the regression of leaf number against Tt accumulation for each sowing date. The phyllochron for each sowing date was also analysed in relation to the photoperiod at emergence. The appearance of axillary leaves and thermal time to axillary leaf production were determined as the point when the number of total leaves exceeded the number of main stem leaves by one. Air temperature (1200 mm) was chosen for phyllochron analysis based on the aerial position of the shoot apical meristem of these top flowering legumes.

3.3 Data analysis

Data for each species were plotted as the reciprocal of the duration (in days) to each development stage (75% germination, 50% emergence and spade leaf) against the mean temperature (T). The inverse of duration (1/days) represents the development rate. For germination, least squares regression analysis was used for both the positive (sub-optimal) and the negative (supra-optimal) linear portion of the response whereby:

Equation 3.4 Development rate = $a_1 + b_1T$ (for the positive linear portion)

Equation 3.5 Development rate = $a_2 + b_2T$ (for the negative linear portion)

The regression coefficients can then be related to T_b, T_{max} and Tt as:

Equation 3.6 $T_b = -a_1/b_1$

Equation 3.7 $T_{max} = -a_2/b_2$

Equation 3.8 $Tt = 1/b_1$ (sub-optimal temperatures)

Equation 3.9 $Tt = -1/b_2$ (supra-optimal temperatures)

The T_{opt} was interpolated from the intersection of the two regression lines as:

Equation 3.10 $T_{opt} = (a_2-a_1)/(b_1-b_2)$

Where the germination rate deviated from a linear model at low or high temperatures, data were excluded from the analysis on the basis that these were outside the species optimal thermal range (Angus *et al.*, 1981). For emergence, first leaf and phyllochron development, least squares regression was analysed only for the positive linear portion of the response (Equation 3.6 and Equation 3.8) because field temperatures did not exceed the optimum, determined from the germination experiment. Where 95% confidence intervals included 0^oC, additional regression analysis was done with T_b set at 0^oC to enable direct comparison of the Tt requirements for each development stage among species and previously published results e.g. Moot *et al.* (2000).

The standard error of T_b and Tt were calculated according to (Campbell *et al.*, 1974) as:

Equation 3.11 $\text{S.E. } T_b = \frac{\bar{y}}{b}\sqrt{\frac{s^2}{N\bar{y}^2} + \left[\frac{\text{S.E.b}}{b}\right]^2}$

Equation 3.12 $\text{S.E. } Tt = \frac{\text{S.E. b}}{b^2}$

Where s^2 is the residual mean square of y, i.e. the development rate and $\bar{y}$ is the sample mean.

Data were analysed using the statistical software Genstat 12.2. Measured variables were analysed using analysis of variance (ANOVA) and the means were separated by least significant difference ($\alpha = 0.05$) tests. For each measured variable, maximum standard errors were reported.

3.4 Results

3.4.1 Germination

3.4.1.1 Final germination percentage

Gompertz functions described the cumulative germination over time for each species (Figure 3.3). Specifically, in each case there was a distinct linear phase before a species by temperature determined final germination percentage. The maximum final germination percentage was above 60% for arrowleaf clover and 80% for the other three species. However, at the higher and lower end of the temperature range the final germination percentage was below these maximum values (Figure 3.4). For example, 'Cefalu' arrowleaf had a final germination percentage of 58 to 71% from 10 to 25°C but this dropped to less than 50% at 5°C and 35°C with no germination at higher temperatures. 'Mihi' Persian had the highest final germination of 90-95% at 30°C but this decreased to 80% at 5°C and 41% at 40°C. Germination was not observed for 'Cefalu' arrowleaf, 'Bolta' balansa or 'Prima' gland clovers at 40°C.

3.4.1.2 Time to 75% germination and germination rate

The number of days to reach 75% of final germination decreased as temperature increased up to 20°C for both Cefalu' arrowleaf and 'Bolta' balansa, 15°C for 'Prima' gland and 30°C for 'Mihi' Persian clover (Figure 3.5). There was then an increase in the duration until the maximum effective temperature of 35°C for both Cefalu' arrowleaf, and 'Bolta' balansa and 40°C for 'Mihi' Persian clovers were reached. For 'Prima' gland clover, the time to germination remained the same from 15 - 20°C before it increased up to the maximum effective temperature of 30°C. The reciprocal of these data was used to define the cardinal temperatures for each species.

In all species, the germination rate showed a positive linear trend from the minimum temperature (T_b) up to the optimum temperature (T_{opt}), and then a negative linear decline until no germination occurred at the maximum temperature (T_{max}) (Figure 3.6). Split-line regression analysis estimated a T_b of 1.4°C for 'Cefalu' arrowleaf, 2.6°C for 'Bolta' balansa, -0.1°C for 'Prima' gland and 4.5°C for 'Mihi' Persian clover. For all species, 95% confidence interval showed T_b estimates for germination included 0°C. 'Cefalu' arrowleaf, 'Bolta' balansa and ‘Prima’ gland clover had a maximum germination rate of between 0.6 – 0.7 seeds per day across a T_{opt} range of 16.2 – 19.7^{o}C and T_{max} of ~34^{o}C. ‘Mihi’ Persian clover had the highest T_{opt} of 32.6^{o}C (1.5 seeds per day) and T_{max} estimate of 45.4^{o}C.

The Tt requirement for 75% of final germination for temperatures in the sub-optimal range was lowest for ‘Mihi’ Persian at 25 (±1.0) ^{o}C d and highest for ‘Cefalu’ arrowleaf at 34 (±0.9) ^{o}C d (Table 3.3). In the supra-optimal range, Tt requirement (from T_{opt} to T_{max}) for ‘Cefalu’ arrowleaf was about three times (25 (±2.0) ^{o}C d) the Tt required for ‘Mihi’ Persian clover (9 (±1.7) ^{o}C d). For ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clover, data points at 37.5 and 40^{o}C were excluded from analysis because they were outside the linear range.

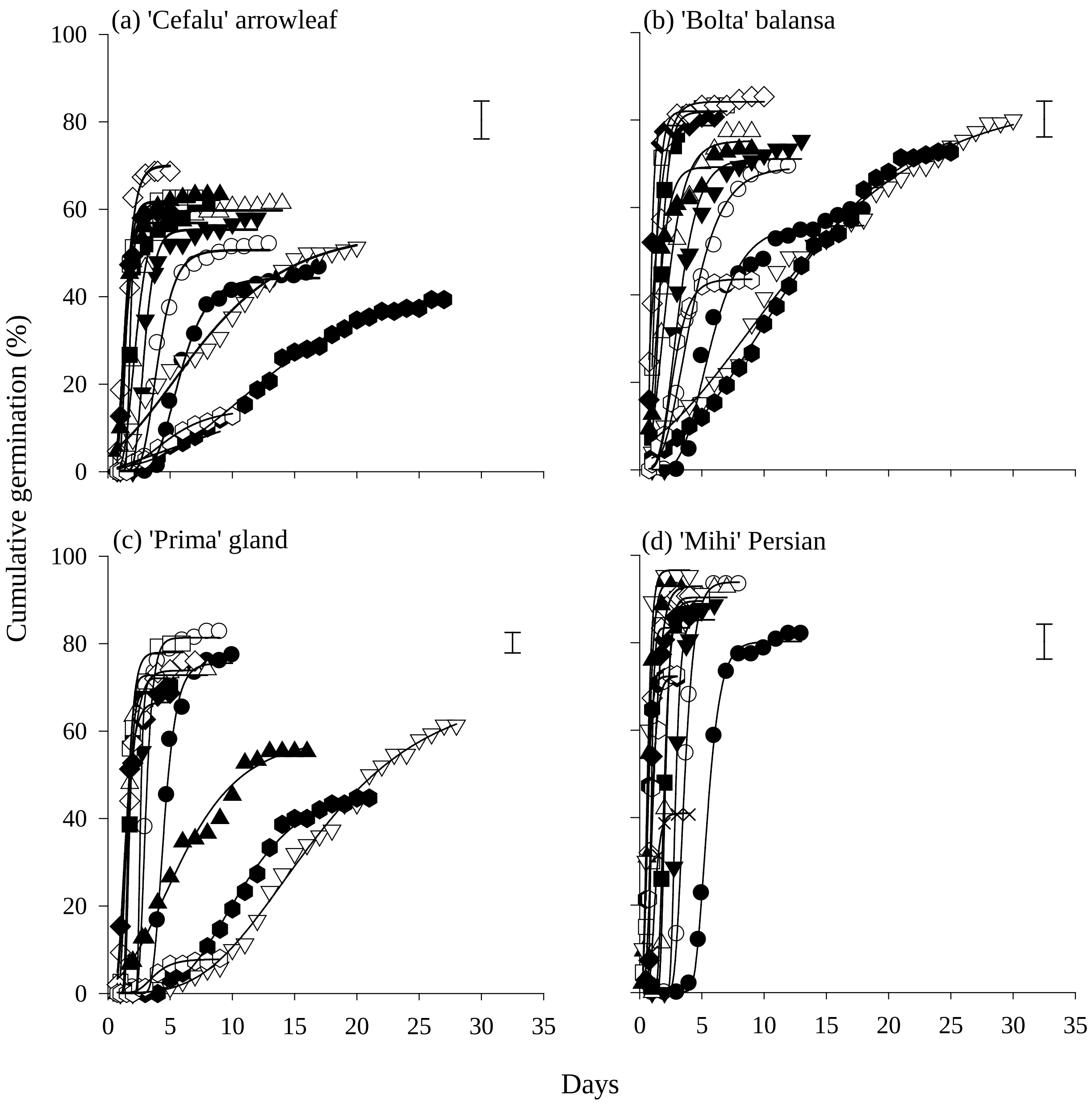


Figure 3.3 Cumulative germination of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers at 13 different constant temperatures. (●) 5°C, (○) 8°C, (▼) 10 °C, (△) 12 °C, (■) 15 °C, (□) 18 °C, (◆) 20 °C, (◇) 22.5 °C, (▲) 25 °C, (▽) 30 °C, (⬢) 35 °C, (⬡) 37.5 °C, (×) 40°C. Error bars represent the maximum standard error of the mean for final germination percentage.

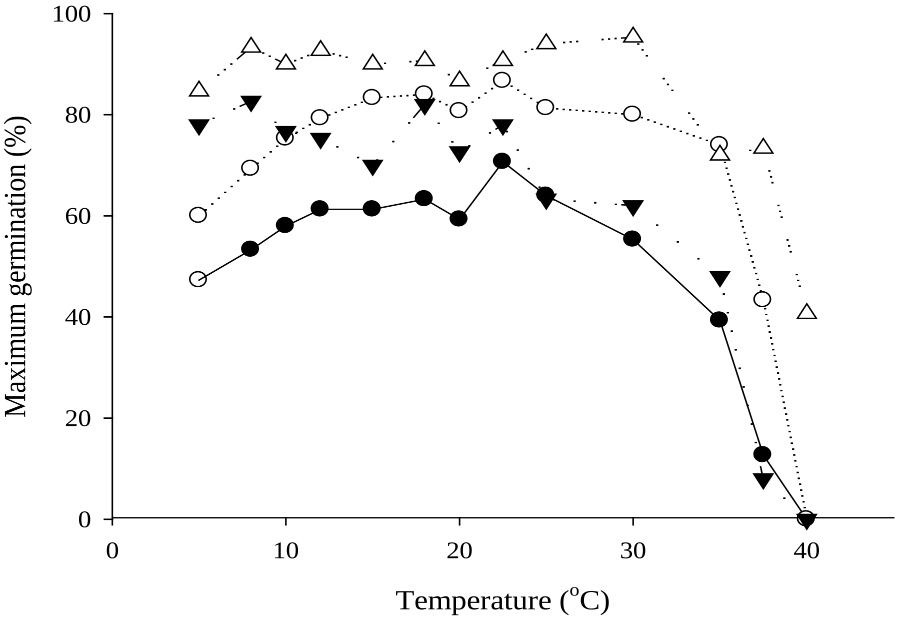


Figure 3.4 Maximum germination (%) for ‘Cefalu’ arrowleaf (●), ‘Bolta’ balansa (○), ‘Prima’ gland (▼) and ‘Mihi’ Persian (△) clovers at different constant temperatures.

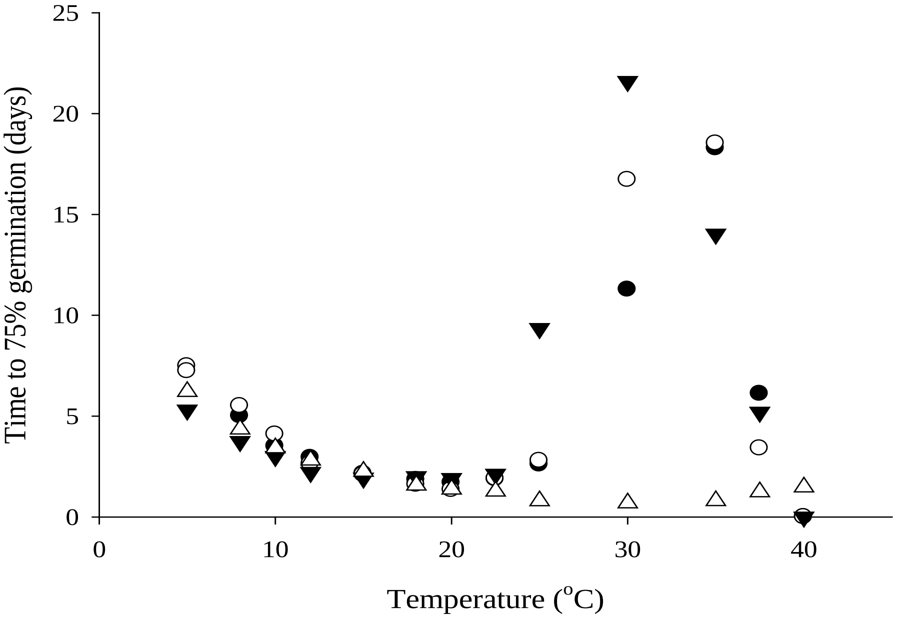


Figure 3.5 Number of days to 75% of final germination for ‘Cefalu’ arrowleaf (●), ‘Bolta’ balansa (○), ‘Prima’ gland (▼) and ‘Mihi’ Persian (△) clovers at different constant temperatures.

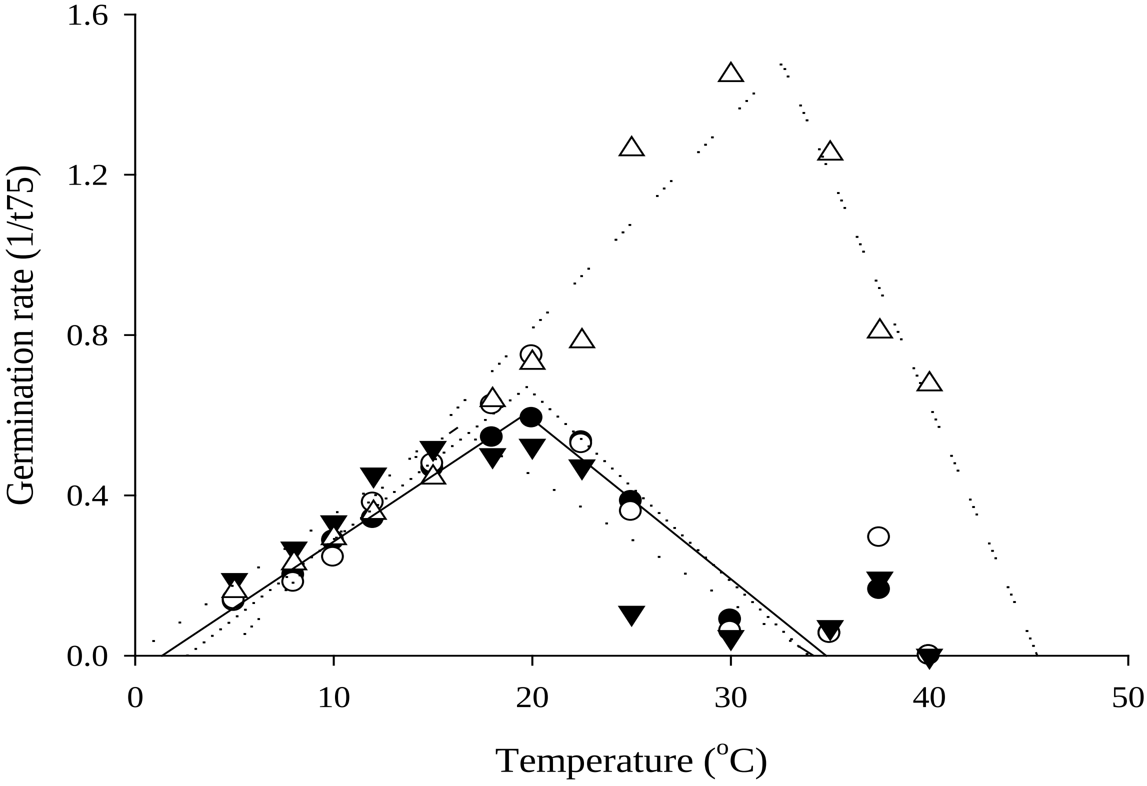


Figure 3.6 Germination rate of 'Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) clovers at different constant temperatures.

Table 3.4 Base (T_b), optimum (T_{opt}) and maximum (T_{max}) temperatures and thermal time (Tt) requirements for 75% germination of four annual clover species.

Species	T_b (°C)	T_{opt} (°C)	T_{max} (°C)	Tt_{sub} (°Cd)	Tt_{sup} (°Cd)	[1]Tt (T_b=0°C) (°Cd)	Excluded temperatures
'Cefalu' a.	1.4	19.7	34.8	30	25	34	37.5, 40
'Bolta' b.	2.6	19.7	33.9	26	21	32	37.5, 40
'Prima' g.	-0.1	16.2	34.1	29	32	28	37.5, 40
'Mihi' P.	4.5	32.6	45.4	19	9	25	_
Max s.e.	0.48	1.22	0.88	1.8	4.9	1.3	
95% c.i.	-5.0, 10.5	13.8, 34.7	25.7, 53.6				

[1] Analysis assumes a base temperature of 0°C. Tt_{sub}, from T_b to T_{opt}; Tt_{sup}, from T_{opt} to T_{max}; a., arrowleaf; b., balansa; g., gland; P., Persian. s.e., standard error; c.i., confidence interval. Coefficients are given in Appendix 1.

3.4.2 Seedling emergence

Gompertz functions also described the cumulative emergence data ($R^2 \geq 85\%$) for each species and sowing date (Figure 3.7). From these Gompertz curves, the date of emergence from each sowing date was calculated using Equation 3.4 as the time when 50% of the final emergence was reached. For example, in all species, seeds sown on 3 June 2010 took 16 days to emerge on 19 June 2010. In contrast, seedlings only took four days to emerge on 23 January 2011 when seeds were sown on 19 January 2011.

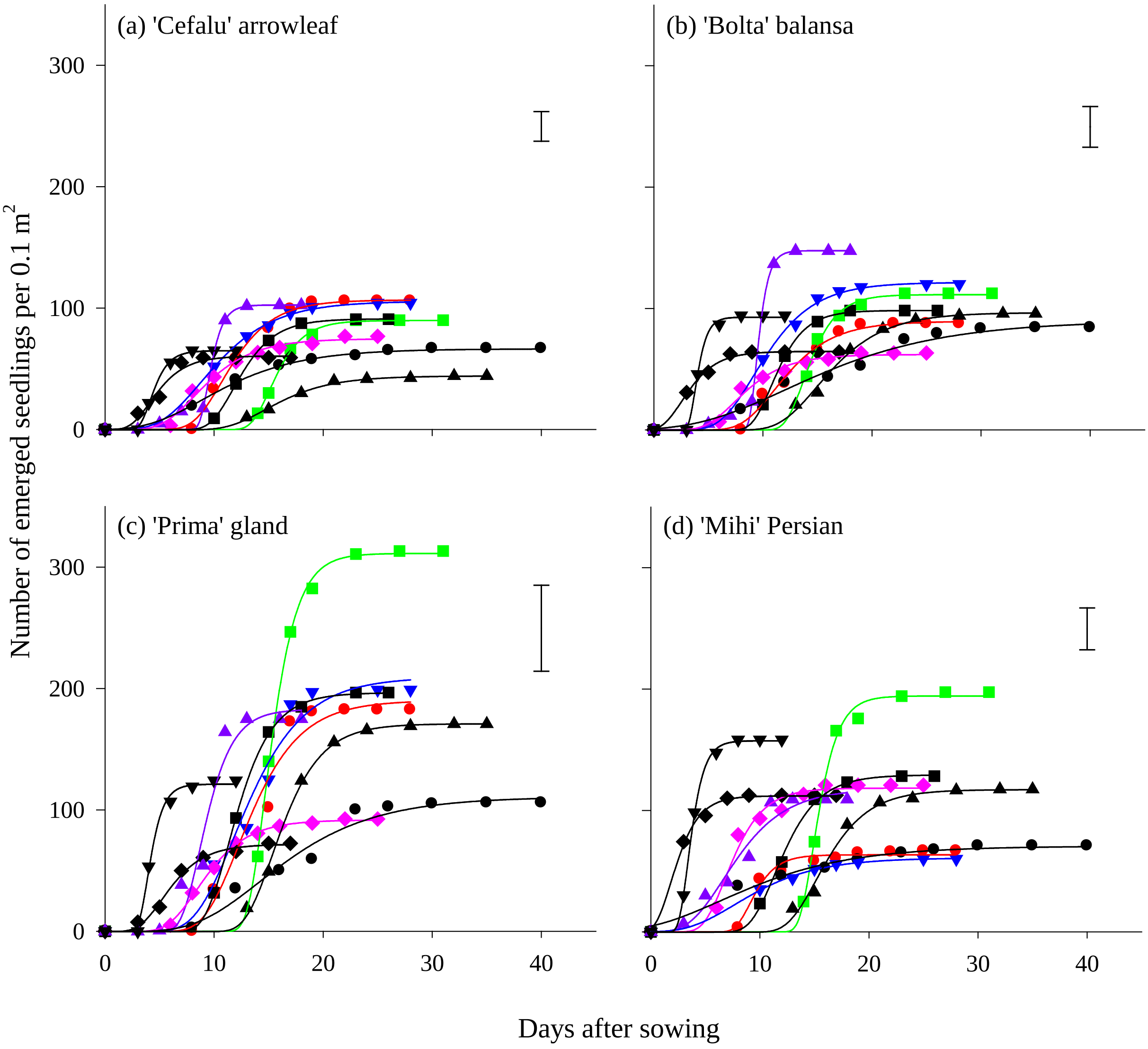


Figure 3.7 Cumulative emergence per 0.1m^2 of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. (●) SD1, (●) SD2, (▼) SD3, (▲) SD4, (■) SD5, (■) SD6, (◆) SD7, (◆) SD8, (▲) SD9, (▼) SD10. Error bars represent the maximum standard error for the final number of emerged seedlings. For date of each SD, refer to Table 3.3.

The number of days to 50% of the final emergence was 16 d at $5.3^{o}C$ for each species, but decreased to 4 d as mean soil temperature (10 mm) increased to $16^{o}C$ (Figure 3.8). The inverse of this relationship was used to establish the rate of emergence against mean soil temperature (Figure 3.9).

In all species, emergence rate as a function of mean soil temperature was described by a linear relationship (Figure 3.9). The T_b calculated for 'Cefalu' arrowleaf was $0.9^{o}C$ and $-0.2^{o}C$ for 'Prima' gland while Tt requirement for 50% emergence was estimated as 82 (±7.4) ^{o}Cd for 'Cefalu' arrowleaf and 97 (±12.5) ^{o}Cd for 'Prima' gland (Table 3.5). For 'Bolta' balansa and 'Mihi' Persian, the rate of emergence versus temperature was explained by a bi-linear relationship (Figure 3.9b).

For 'Bolta' balansa, at temperatures between 5.1 to 10.4 ^{o}C, T_b and Tt were estimated as -4.6 ^{o}C and 145 (±26.1) ^{o}C d. As temperature rose to $18.8^{o}C$, emergence rate increase significantly and Tt required for emergence was only 37 (±12.2) ^{o}C d with a T_b extrapolated of $7.2^{o}C$. Similarly, in 'Mihi' Persian, seedlings took longer to emerge (111 (±16.5) ^{o}C d, T_b of $-1.8^{o}C$) at a mean soil temperature between 5.2 to $12.1^{o}C$, but when temperature increased further to $18.5^{o}C$, seedlings only took 19 (±10.2) ^{o}C d to emerge at an extrapolated T_b of $10.7^{o}C$. In all species, 95% confidence intervals for T_b analysis encompass $0^{o}C$ and when T_b was set at $0^{o}C$, Tt requirements for emergence were similar (Table 3.5).

In all species, data points at ~14, 18 and $21^{o}C$ were excluded from the regression analysis (in Figure 3.9) due to water stress in the field which crusted the soil surface and delayed emergence of seedlings (Plate 2).

Plate 2 Dry, cracked and crusted soil surface due to water stress in Iversen 9 field, Lincoln University.

Table 3.5 Base temperature (Tb) and thermal time (Tt) requirements for 50% emergence of ‘Cefalu’ arrowleaf , ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Iversen 9 field, Lincoln University, New Zealand.

Species	Temperature range (^oC)	T_b (^oC)	Tt (^oCd)	[1]Tt ($T_b=0^oC$) (^oCd)
‘Cefalu’ arrowleaf	5.3 - 19.1	0.9	82	88
‘Bolta’ balansa	5.1 - 10.4	-4.6	145	92
	12.1 - 18.8	7.2	37	67
‘Prima’ gland	5.2 - 18.8	-0.2	97	96
‘Mihi’ Persian	5.2 - 12.1	-1.8	111	93
	16.1 - 18.5	10.7	19	50
Max. s.e.		1.01	26.12	7.24
95% c.i.		-19.1, 21.4		

[1] Analysis assumes a base temperature of 0^oC. s.e., standard error; c.i., confidence interval. Coefficients are given in Appendix 2.

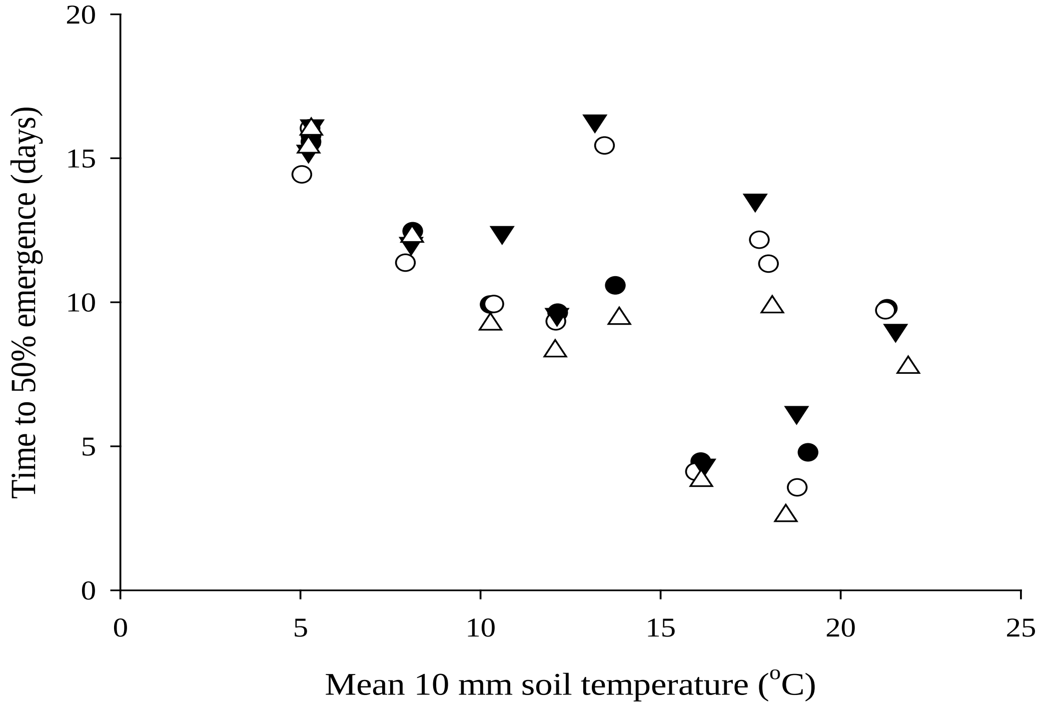


Figure 3.8 Number of days to 50% of final emergence of 'Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) at different mean soil temperatures (10 mm) in Iversen 9 field, Lincoln University, New Zealand.

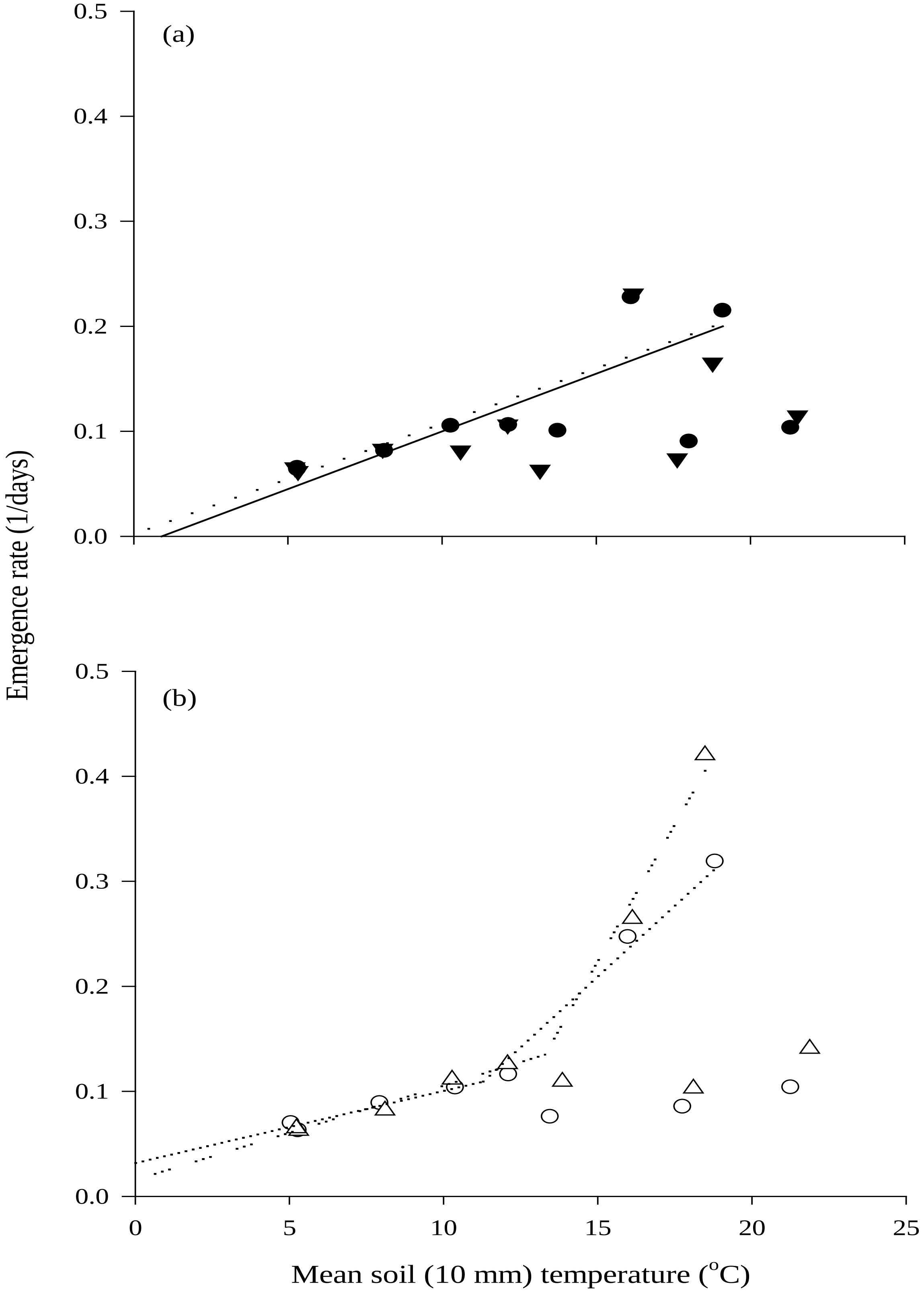


Figure 3.9 Emergence rate of Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) clover at different mean soil (10 mm) temperatures in Iversen 9 field, Lincoln University, New Zealand.

3.4.3 First leaf appearance

The number of days from sowing to first leaf appearance (spade leaf) was ~41 days at 5.3^{o}C for each species and gradually decreased to ~11 days as mean soil (10 mm) temperature increased to 18.6^{o}C (Figure 3.10a). Linear regression between the rate of first leaf appearance and mean soil (10 mm) temperature (Figure 3.10b) enabled T_b and Tt to be calculated (Table 3.6). Note, the sowing dates that did not conform with the predicted model for emergence due to soil surface crusting, followed a similar pattern for first leaf appearance (Figure 3.10b).

In all species, T_b calculated was not different from 0^{o}C. Therefore, when T_b was set to 0^{o}C, the Tt requirement for first leaf appearance averaged 187 (±7.3) ^{o}C d for both 'Bolta' balansa and 'Prima' gland, compared with 191 (±5.6) ^{o}C d for 'Mihi' Persian and 215 (±4.9) ^{o}C d for 'Cefalu' arrowleaf.

Table 3.6 Base temperature (T_b) and thermal time (Tt) requirements for 50% first leaf appearance of 'Cefalu' arrowleaf , 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown in Iversen 9 field, Lincoln University, New Zealand.

Species	T_b (^{o}C)	Tt (^{o}C d)	R^2 (%)	[1]Tt (T_b=0^{o}C) (^{o}C d)
'Cefalu' arrowleaf	1.3	193	94	215
'Bolta' balansa	1.6	166	86	189
'Prima' gland	2.6	143	92	185
'Mihi' Persian	1.8	166	92	191
Maximum s.e.	0.38	12.8		7.3
95% c.i.	-4.4, 6.9			

[1]Analysis assumes a base temperature of 0^{o}C. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

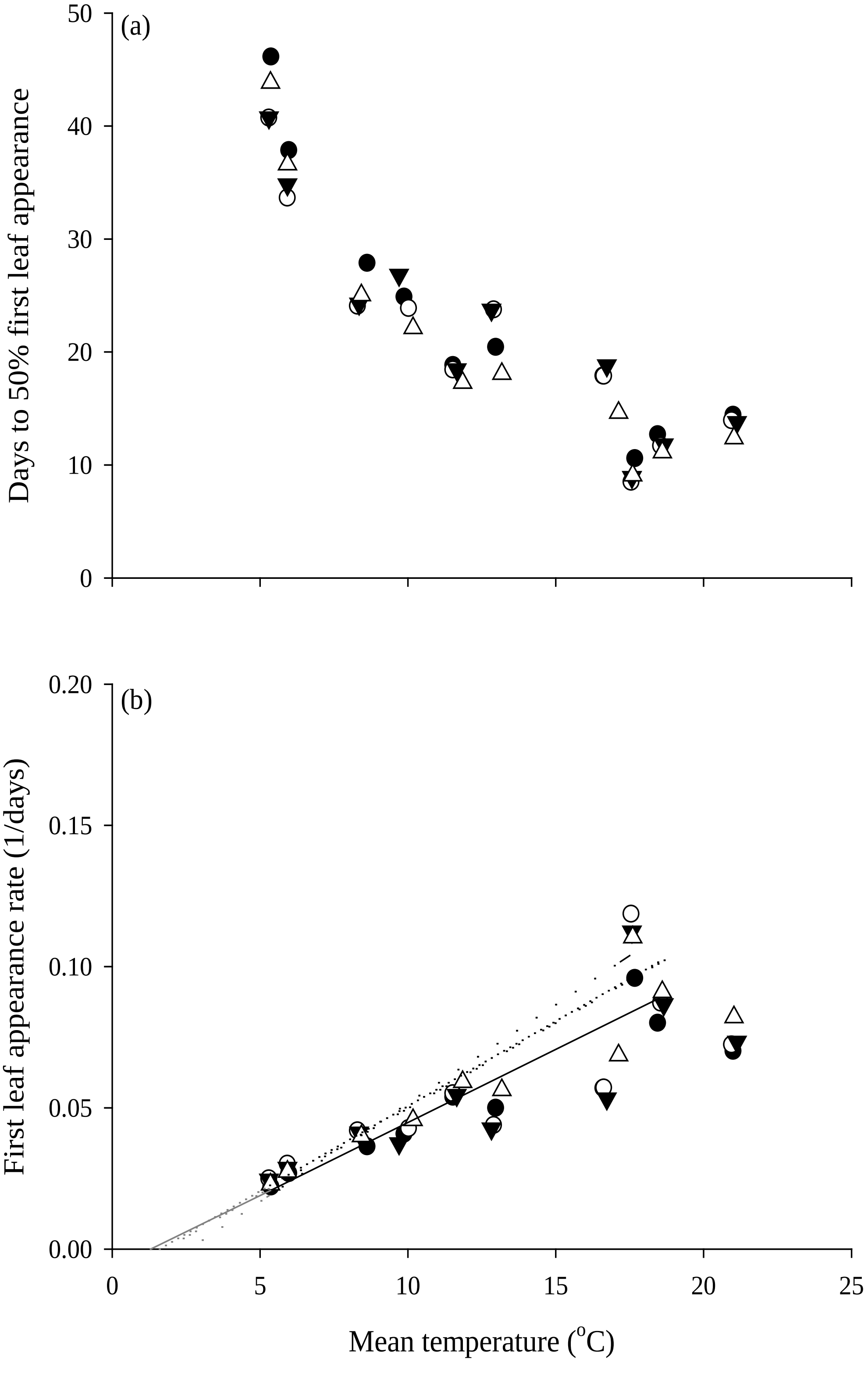


Figure 3.10 Number of days from sowing to first leaf appearance (a) and first leaf appearance rate (b) of Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) clovers at different mean soil (10 mm) temperatures in Iversen 9 field, Lincoln University, New Zealand. Gray lines are extrapolated lines.

3.4.4 Phyllochron

In all species, the appearance of leaves on the main stems increased linearly with thermal time accumulation (Figure 3.11). The slope of each linear relationship was used to calculate the phyllochron (^{o}C d/leaf) for each sowing date. In each species, there was an interaction ($P<0.001$) between sowing date and species for the phyllochron (Table 3.7). The phyllochron exhibited a declining trend following each successive sowing date with the highest phyllochron when sown on 26 February 2010.

In each species, the lowest phyllochron was 53 (±1.5) ^{o}C d/leaf for 'Cefalu' arrowleaf, 44 (±1.1) ^{o}C d/leaf for 'Bolta' balansa, 33 (±1.6) ^{o}C d/leaf for 'Prima' gland and 61 (±0.9) ^{o}C d/leaf for 'Mihi' Persian clovers when the seeds were sown on 9 November 2010. The variation in phyllochron with sowing time suggests a secondary effect at seedling emergence. To account for this variation, the effect of photoperiod was investigated.

Table 3.7 Main stem phyllochron (^{o}C d/leaf) of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown at different dates in Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Species			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26/2/2010	116	82	91	93
30/3/2010	96	77	87	93
4/5/2010	90	70	85	87
3/6/2010	83	70	74	78
7/7/2010	91	69	65	82
14/8/2010	80	55	50	77
25/9/2010	63	49	41	68
9/11/2010	53	44	33	61
20/12/2010	60	44	33	67
19/1/2011	62	46	36	67
	SD	Species	SD*Species	
P- value	<0.001	<0.001	<0.001	
S.E.M.	1.3	0.6	2.2	
Except when comparing means with the same level of SD			2.0	
L.S.D. (5%)	3.7	1.8	6.1	
Except when comparing means with the same level of SD			5.7	

Note: Thermal time used air temperature ($T_b = 0^{o}C$) and calculated starting from first leaf appearance.
SD, Sowing date. S.E.M. Standard error of mean; L.S.D., Least significant differences.

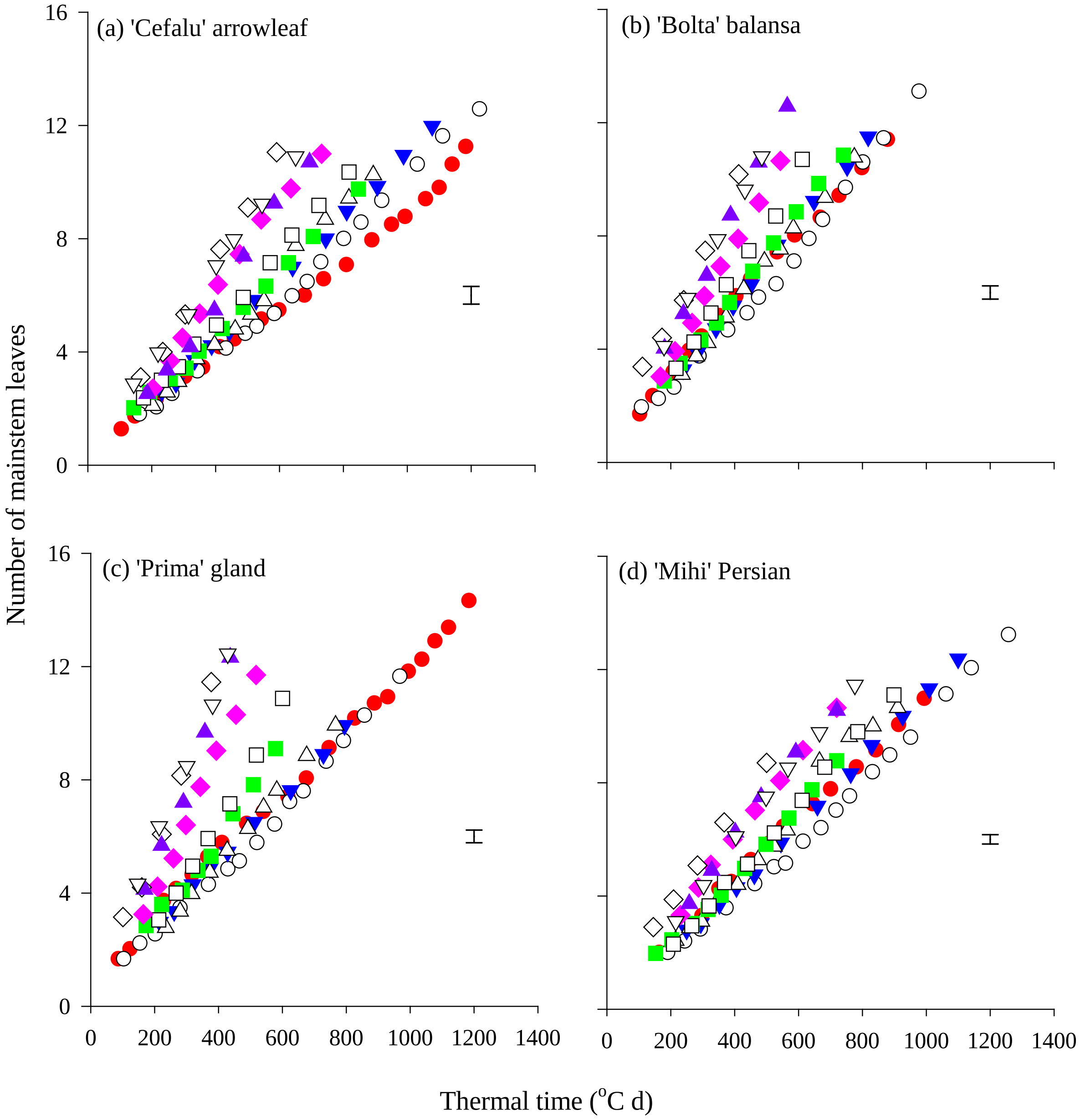


Figure 3.11 The number of leaves on the mainstem of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on 10 dates (SD) at Lincoln University, Canterbury, New Zealand. SD1(●), SD2 (○), SD3 (▼), SD4 (△), SD5 (■), SD6 (□), SD7 (◆), SD8 (◇), SD9 (▲), SD10(▽). Note: Thermal time used air temperature (T_b = 0°C) and calculated starting from first leaf appearance. Error bars represent maximum standard error for the final number of leaves. See Table 3.3 for sowing dates.

3.4.4.1 Phyllochron in relation to photoperiod at emergence

When photoperiod was analysed, it was found that the phyllochron responded to changes in the daylength and direction of photoperiod at seedling emergence and formed a hysterisis (Figure 3.12). In each species, when seedlings emerged following the shortest day (21 June) into an increasing photoperiod up to 16 hours, the phyllochron decreased by 6 (±0.9) ^{o}C d leaf^{-1} hour^{-1} for ‘Cefalu’ arrowleaf, 5 (±0.9) ^{o}C d leaf^{-1} hour^{-1} for ‘Bolta’ balansa, 7 (±1.1) ^{o}C d leaf^{-1} hour^{-1} for ‘Prima’ gland and 3 (±0.6) ^{o}C d leaf^{-1} hour^{-1} for ‘Mihi’ Persian clover (Table 3.8). Following the longest day, as photoperiod shortened to ~13 hours in late February, phyllochron rose rapidly by 21 (±2.6) ^{o}C d leaf^{-1} hour^{-1} for both ‘Cefalu’ arrowleaf and ‘Prima’ gland, 14 (±1.4) ^{o}C d leaf^{-1} hour^{-1} for ‘Bolta’ balansa and 10 (±1.0) ^{o}C d leaf^{-1} hour^{-1} for ‘Mihi’ Persian clover. However, as photoperiod continued to decrease further from early autumn into the shortest day in winter, the phyllochron decreased by 9 (±1.5) ^{o}C d leaf^{-1} hour^{-1} for ‘Cefalu’ arrowleaf , 4 (±0.9) ^{o}C d leaf^{-1} hour^{-1} for both ‘Bolta’ balansa and ‘Prima’ gland and 7 (±0.9) ^{o}Cd leaf^{-1} hour^{-1} for and ‘Mihi’ Persian clover.

Table 3.8 Coefficients of fitted relationship between phyllochron (^{o}Cd/leaf) and direction of photoperiod (hours) change in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Iversen 9 field, Lincoln University, New Zealand.

Species	Direction of photoperiod	Relationship	R^2 (%)
‘Cefalu' arrowleaf	Increase	y = 143.3 - 5.73x	93
	Decrease into autumn	y = 400.0 – 20.80x	86
	Decrease into winter	y = -5.0 + 8.72x	68
‘Bolta' balansa	Increase	y = 116.2 - 4.77x	88
	Decrease into autumn	y = 268.7 – 13.72x	90
	Decrease into winter	y = 33.1 + 3.63x	55
‘Prima' gland	Increase	y = 136.8 - 6.84x	90
	Decrease into autumn	y = 368.5 – 20.51x	95
	Decrease into winter	y = 37.3 + 4.08x	56
‘Mihi' Persian	Increase	y = 114.5 - 3.39x	91
	Decrease into autumn	y = 227.9 – 9.85x	89
	Decrease into winter	y = 13.0 + 6.67x	83

R^2, coefficient of determination.

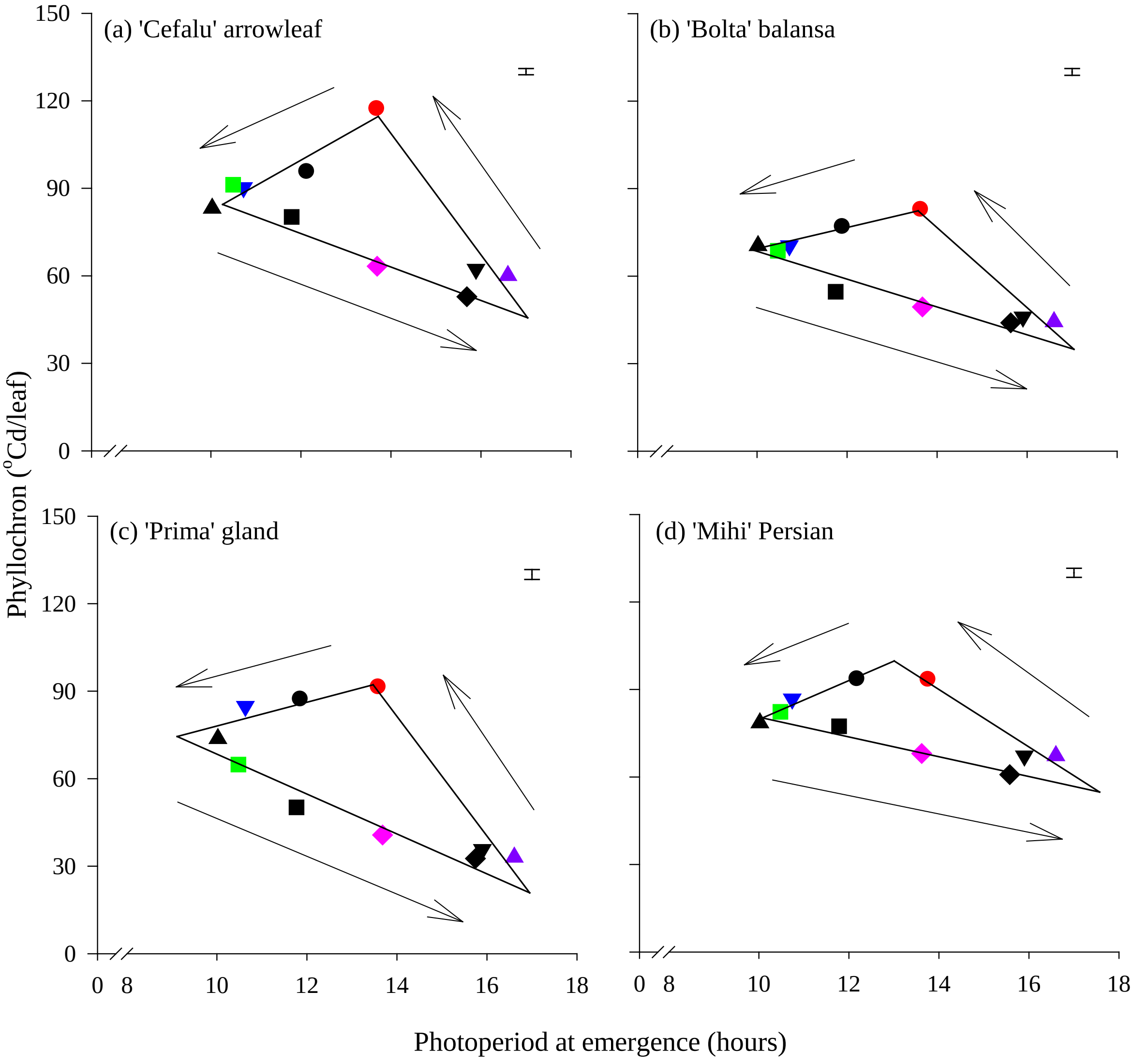


Figure 3.12 Phyllochron in response to photoperiod at emergence of (a)'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown on 10 dates in Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). For actual sowing dates (SD) see Table 3.3.

3.4.5 Appearance of axillary leaves

The first axillary leaf was initiated in the axil of the first (spade) leaf in ‘Bolta’ balansa and ‘Mihi’ Persian clovers and in the axil of the second (first trifoliate) leaf in ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers (Plate 3 - Plate 6). The development of axillary leaves led to an exponential increase in the total number of leaves, whereas the number of leaves on the main stem continued to increase linearly with time (Figure 3.13).

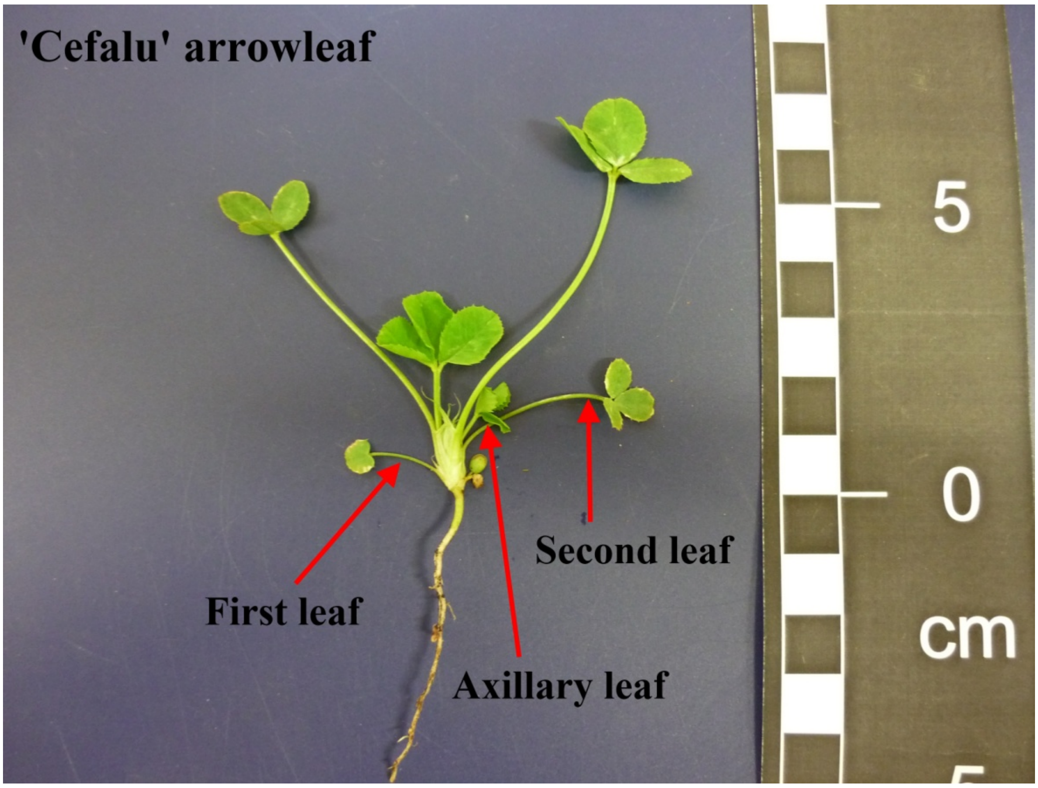


Plate 3 The initiation of the first axillary leaf in the axil of the second (first trifoliate) leaf of ‘Cefalu’ arrowleaf clover.

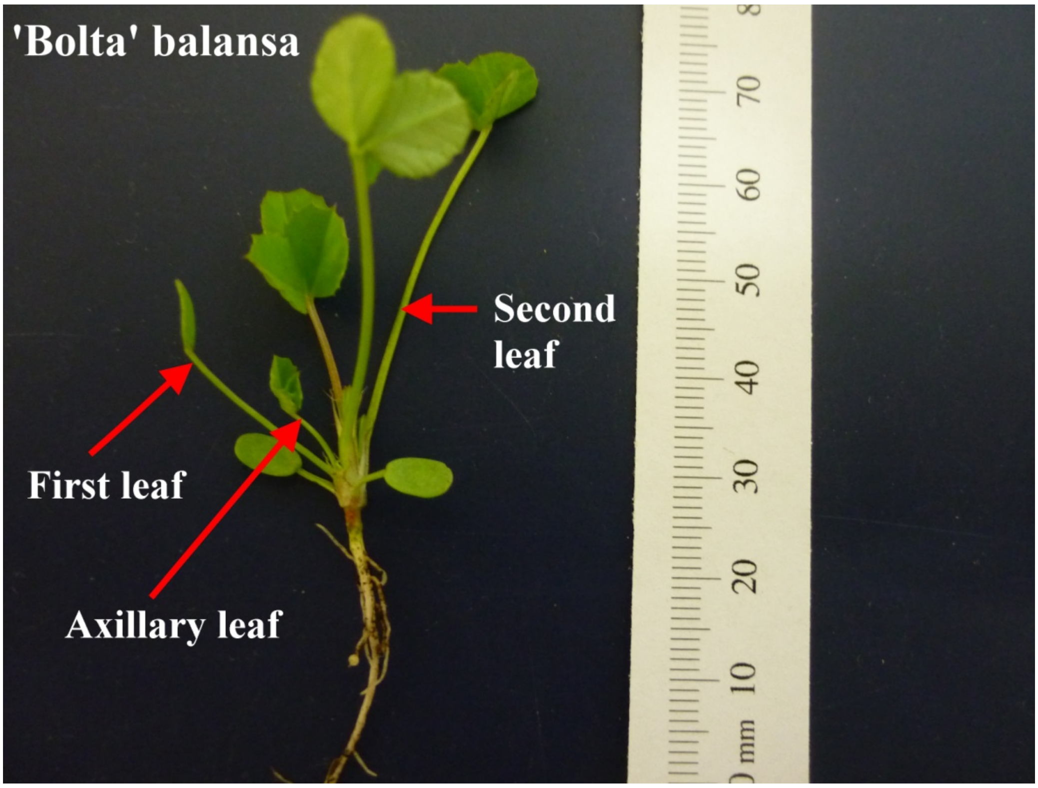


Plate 4 The initiation of the first axillary leaf in the axil of the first (spade) leaf of 'Bolta' balansa clover.

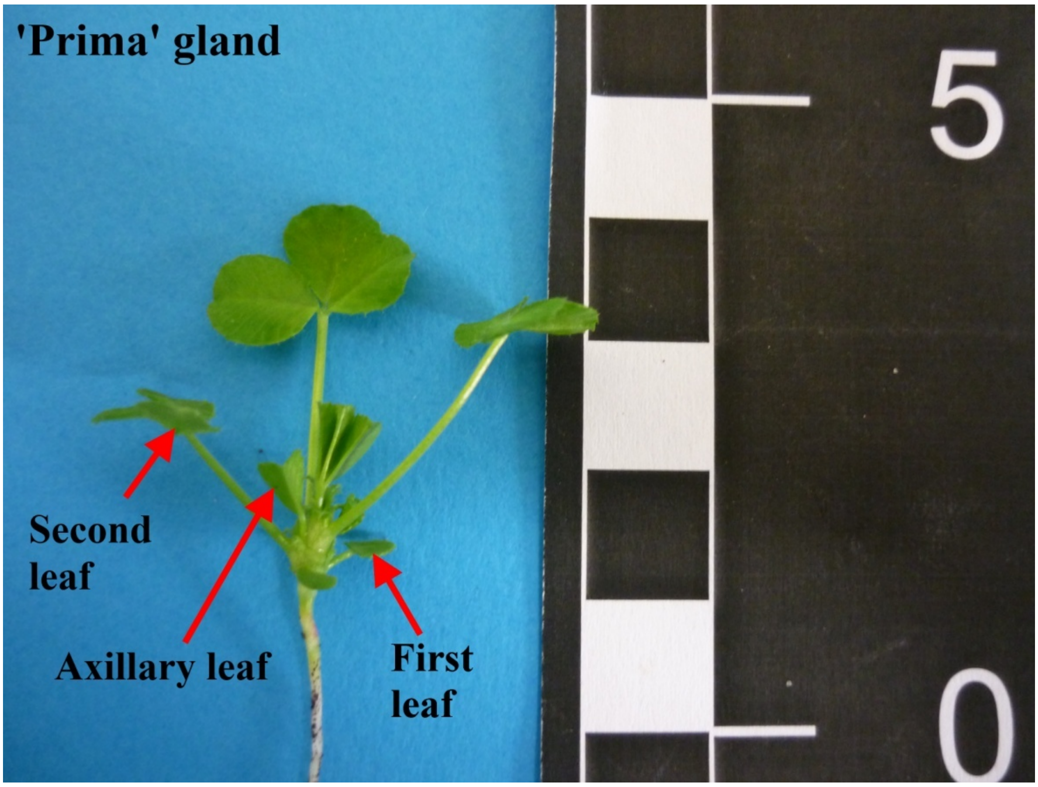


Plate 5 The initiation of the first axillary leaf in the axil of the second (first trifoliate) leaf of 'Prima' gland clover.

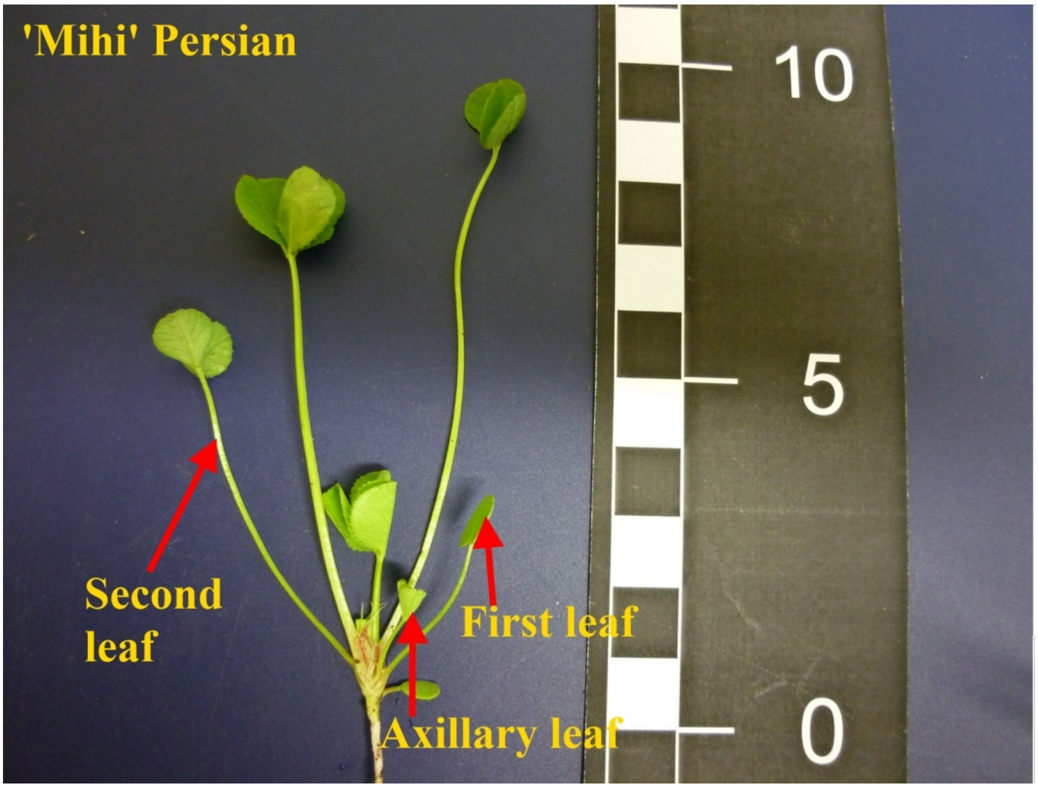


Plate 6 The initiation of the first axillary leaf in the axil of the first (spade) leaf of ‘Mihi’ Persian clover.

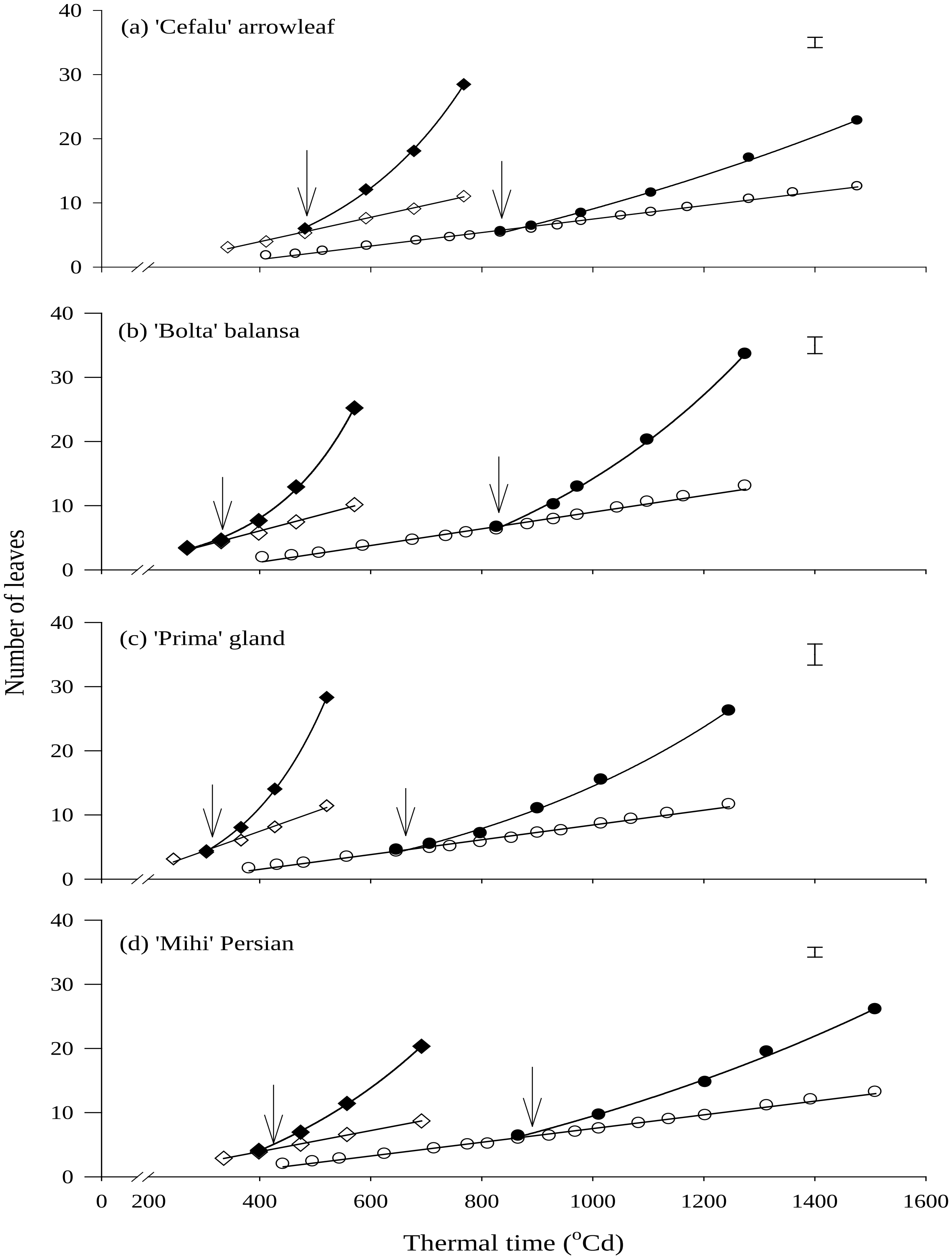


Figure 3.13 Number of total (closed symbols) and main stem (open symbol) leaves of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clover plotted against thermal time after sowing (T_b = 0°C, air temperature) of SD2(●) and SD8(◆). See Table 3.3 for actual sowing date (SD). Arrows indicate time of axillary leaf appearance. Error bars represent the maximum standard error for the final total leaf number. See Appendix 3 for results of the other eight sowing dates.

3.4.5.1 Initiation of axilary leaf at different sowing dates

The time to axillary leaf appearance data indicated a species by sowing date interaction ($P<0.018$) (Table 3.9). For example, in 'Prima' gland clover, the thermal time requirement from sowing to axillary leaf appearance was 642 (±49.1) ^{o}C d when sown on 30th March 2010 (SD2), but it only took 320 (±15.6) ^{o}C d (which is half of the Tt for SD2) to produce the first axillary leaf when seeds were sown on 9th November 2010 (SD8) (Figure 3.13c). When the number of leaves on the main stem was compared with axillary leaf appearance (Table 3.10), SD2 had four leaves whereas SD8 had five leaves. This one leaf difference was 322 ^{o}C d in terms of the thermal time requirement. The variation of phyllochron in response to photoperiod affected the time to axillary leaf appearance (Table 3.11). The hysteresis response between the time of first axillary leaf and direction of photoperiod (Figure 3.14) followed the same pattern as phyllochron (Figure 3.12).

Table 3.9 Thermal time requirement (^{o}Cd) from sowing to the appearance of the first axillary leaf for 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on 10 dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

	Species			
SD	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26/2/2010	891	501	534	592
30/3/2010	774	781	642	806
4/5/2010	737	571	568	655
3/6/2010	767	540	563	643
7/7/2010	794	488	475	633
14/8/2010	736	449	442	549
25/9/2010	505	438	413	463
9/11/2010	429	300	320	436
20/12/2010	609	404	411	490
19/1/2011	477	352	287	442

	SD	Species	SD*Species
P- value	<.001	<.001	0.018
S.E.M.	25.8	13.1	44.3
Except when comparing means with the same level of SD			41.5
L.S.D. (5%)	74.9	36.9	124.0
Except when comparing means with the same level of SD			116.6

Thermal time quantified based on air temperature ($T_b=0^{o}C$). SD, Sowing date. S.E.M. Standard error of mean; L.S.D., Least significant differences.

Table 3.10 Number of leaves on the main stem to axillary leaf appearance for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on 10 dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

	Species			
SD	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26/2/2010	5.5	3.9	4.6	4.4
30/3/2010	5.1	5.9	4.4	5.0
4/5/2010	5.3	4.7	4.1	4.4
3/6/2010	5.2	4.4	4.1	4.1
7/7/2010	5.0	3.8	3.8	4.3
14/8/2010	6.0	3.8	3.5	3.8
25/9/2010	4.5	4.4	4.5	4.1
9/11/2010	4.3	3.9	4.7	4.4
20/12/2010	5.6	4.6	6.2	4.0
19/1/2011	5.3	5.4	4.9	4.4

	SD	Species	SD*Species
P- value	>0.05	<.001	<0.01
S.E.M.	0.24	0.12	0.40
Except when comparing means with the same level of SD			0.37
L.S.D. (5%)	0.70	0.33	1.13
Except when comparing means with the same level of SD			1.05

SD, Sowing date. S.E.M. Standard error of mean; L.S.D., Least significant differences.

Table 3.11 Coefficients of fitted relationship between time to axillary leaf appearance (^{o}Cd) and direction of photoperiod (hours) change in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clover.

Species	Direction of photoperiod	Relationship	R^2 (%)
‘Cefalu' arrowleaf	Increase	y = 1444 - 65.3x	91
	Decrease into autumn	y = 2484 – 119.0x	45
	Decrease into winter	y = 479 + 28.2x	51
‘Bolta' balansa	Increase	y = 888 - 36.1x	86
	Decrease into autumn	y = 1703 – 82.2x	78
	Decrease into winter	y = -820 + 133.3x	87
‘Prima' gland	Increase	y = 777 - 28.3x	92
	Decrease into autumn	y = 1198 – 47.8x	97
	Decrease into winter	y = 102 + 45.1x	85
‘Mihi' Persian	Increase	y = 1038 - 40.1x	93
	Decrease into autumn	y = 1677 – 74.9x	81
	Decrease into winter	y = -172 + 79.5x	86

R^2, coefficient of determination.

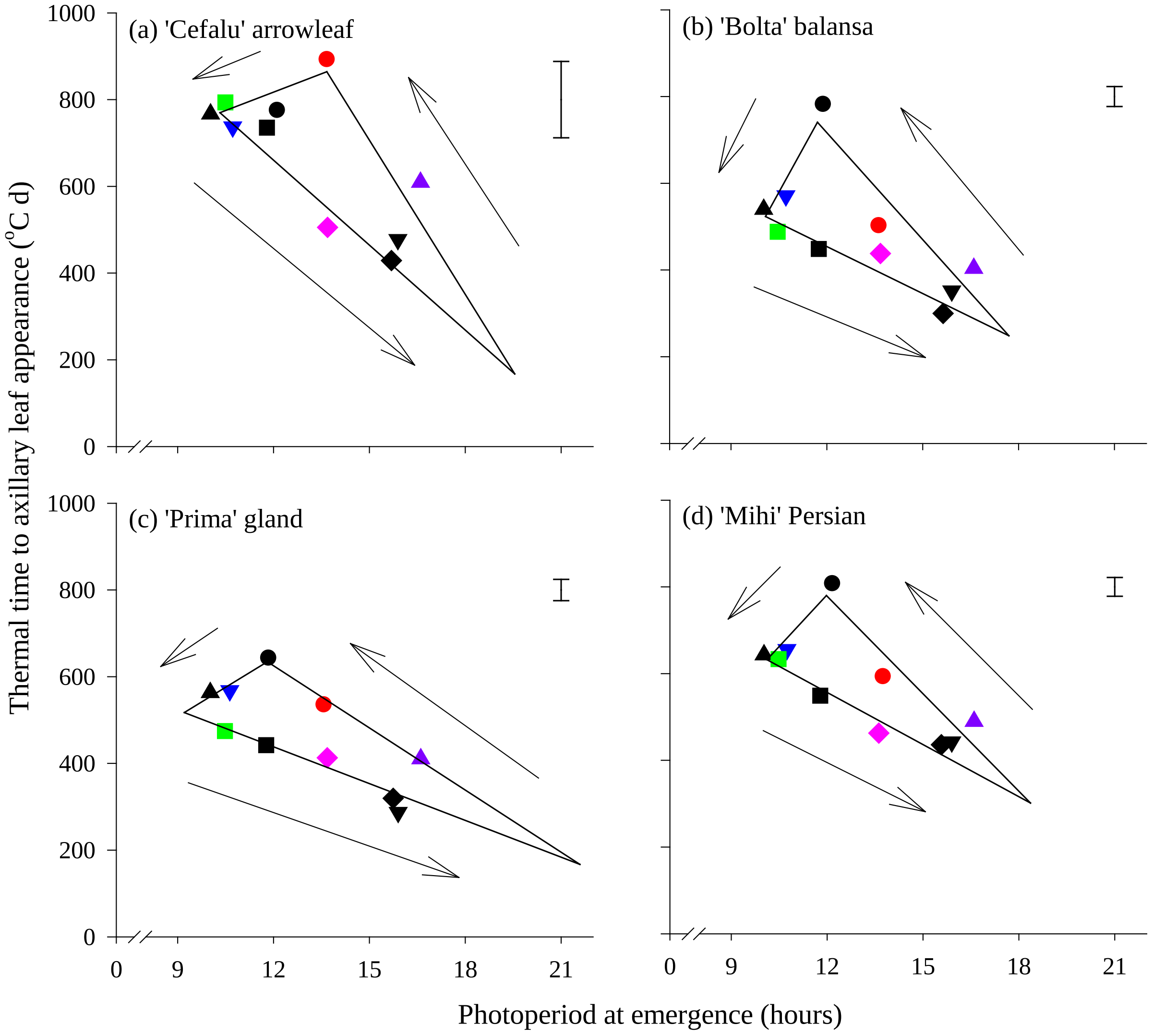


Figure 3.14 Thermal time to axillary leaf appearance against photoperiod at emergence of (a)‘Cefalu’ arrowleaf, (b)‘Bolta’ balansa, (c)’Prima’ gland and (d)’Mihi’ Persian clover sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). Error bars represent the maximum standard error of the mean. See Table 3.3 for actual sowing date (SD).

3.5 Discussion

3.5.1 Phyllochron

Leaf appearance was found to be constant throughout the vegetative growth period within each sowing date but differed among sowing dates (Figure 3.11). For each sowing date, leaf appearance was linearly (R^2>98%) related to thermal time accumulation based on air temperature, but the slope of each relationship differed among sowing dates. The factor contributing to this variation is either the effect of photoperiod at seedling emergence or a systematic error in the calculation of thermal time (Jamieson *et al.*, 1995a).

The systematic variation in phyllochron from autumn to spring sowing, with an increase for summer (Table 3.7) implies that the production of leaves on the main stem was influenced by a secondary factor. The hysteresis with photoperiod suggests that accurate prediction of leaf appearance for these annual clovers required consideration of the photoperiod at the location of planting. In this experiment, the phyllochron decreased with increased photoperiod up to 16 hours and rose as photoperiod shortened to ~13 hour into the autumn. It decreased again as photoperiod continued to shorten further into the winter (Figure 3.12). Similar results were reported for lucerne (Brown *et al.*, 2005), winter wheat (Baker *et al.*, 1980) and oats (Sonego, 2000) in response to photoperiod at emergence. Cao and Moss (1989) found that the phyllochron of winter wheat and spring barley were affected by the changes of photoperiod throughout the growing period but did not support the hypothesis that the phyllochron is determined by the photoperiod at seedling emergence.

The phyllochron for 'Bolta' balansa clover ranged from 82 (±1.1)^{o}C d/leaf at 13.6 hour photoperiod to 44 (±0.2) ^{o}C d/leaf at 15.6 hour photoperiod when calculated with air temperature above a T_b of 0^{o}C. In contrast, Monks (2009) estimated a single phyllochron of 47 ^{o}C d/leaf (using air temperature, T_b = 0^{o}C) for 'Bolta' balansa clover sown at six different dates in a field experiment at Lincoln University, New Zealand. However, the limited number of sowing dates did not allow him to test the relationship between phyllochron and photoperiod.

Despite reports of the variation of phyllochron with photoperiod, leaf appearance rate is often reported to be independent of photoperiod and sowing time (Hotsonyame and Hunt, 1997; Jamieson *et al.*, 1995a; Miglietta, 1989; Slafer and Rawson, 1997). Its production is considered to be mainly driven by temperature perceived by the meristem apex (Peacock, 1975). Jamieson *et al.* (1995a) suggested that the discrepancies of leaf appearance in wheat with sowing dates often occur when the thermal time was quantified based on air temperature when the apex was below ground. They found that by using near-surface soil temperature of the shoot apex, the leaf appearance rate was constant across sowing dates and therefore concluded that soil temperature was the best predictor of leaf appearance in wheat. However, Sonego (2000) reported that the phyllochron in oats was not constant among sowing dates despite using soil temperature to measure leaf production.

Peacock (1975) stated that knowing the precise location of the meristem apex (growing point) is critical to determine whether soil or air temperature will significantly affect leaf production (Section 2.6.1). In this case, the use of near-surface soil temperature was suitable to measure the leaf appearance rate in wheat (Jamieson *et al.*, 1995a) because the apex in cereal crops remained below the ground until flower initiation began (Scott and Hines, 1991; Sonego, 2000). However, the use of soil temperature is not biologically accurate to determine leaf appearance in these annual legumes because the apex of the stem was raised above the soil as it elongates, exposing the apex above the newest axil of the branch .

In this study, both soil and air temperatures were recorded to analyse the leaf appearance in annual clovers. Both methods showed a strong linear (R^2>98%) relationship between the leaf appearance and thermal time accumulation but when compared, there was no difference (P=0.358) between the R^2 of both temperatures (Table 3.12). Importantly, the phyllochron quantified based on soil temperature still differed (P<0.001) with sowing date (Table 3.13). Therefore, based on the location of the stem apex, air temperature was chosen to measure the leaf appearance and phyllochron of annual clovers.

Table 3.12 The coefficient of determination (R^2) of relationship between the leaf appearance and thermal time accumulation based on air and soil temperatures.

	Species			
Temperature	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
Air	99.54	99.61	99.28	99.53
Soil	99.50	99.43	99.10	99.54
	Temperature	Species	Temperature*Species	
P- value	0.358	0.064	0.886	
S.E.M.	0.074	0.105	0.149	

SD, Sowing date. S.E.M. Standard error of mean.

Table 3.13 The phyllochron (^{o}C d/leaf, based on 10 mm soil temperature) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown at different dates in Iversen 9 field, Lincoln University, New Zealand.

	Species			
SD	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26/2/2010	119	82	94	93
30/3/2010	100	80	91	98
4/5/2010	96	75	90	92
3/6/2010	92	76	80	88
7/7/2010	102	76	69	92
14/8/2010	93	64	56	97
25/9/2010	60	52	40	85
9/11/2010	49	39	27	76
20/12/2010	56	40	29	81
19/1/2011	64	41	30	82
	SD	Species	SD*Species	
P- value	<0.001	<0.001	<0.001	
S.E.M.	1.3	0.7	2.3	
Except when comparing means with the same level of SD			2.2	
L.S.D. (5%)	3.8	1.9	6.4	
Except when comparing means with the same level of SD			6.0	

SD, Sowing date. S.E.M. Standard error of mean; L.S.D., Least significant differences.

3.5.2 Axillary leaf appearance

In each species, the time to axillary leaf initiation differed with sowing dates (Table 3.9) and responded to changes in direction of photoperiod at seedling emergence (Figure 3.14). The number of leaves on the main stem at the time of the first axillary leaf was affected ($P<0.01$) by a species by sowing date interaction (Table 3.10). In most of the sowing dates, the initiation of axillary leaf began after the appearance of four leaves on the main stem of 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers and five leaves on the main stem of 'Cefalu' arrowleaf clover. Leaf appearance on the main stem and its phyllochron were driven by the accumulation of thermal time and photoperiod at seedling emergence. Therefore, the differences in timing of axillary leaf appearance among sowing dates were caused by the differences in the phyllochron at sowing. For example, the phyllochron in 'Prima' gland clover was 87 (±0.5) ^{o}C d/leaf when sown on 30th March 2010 (SD2) compared with 33 (±0.1) ^{o}C d/leaf when sown on 9th November 2010 (SD8) (Table 3.7). The longer phyllochron in SD2 plants caused a longer time of 642 ^{o}C d to produce its first axillary leaf after the appearance of four leaves on the main stem. In contrast, November sown plants only required 320 ^{o}C d to initiate axillary leaves after five leaves had appeared on the main stem (Section 3.4.5.1). Because the photoperiod at seedling emergence affected the phyllochron, this was the main cause of variation in timing to axillary leaf production. Therefore, the time of seedling emergence is important in predicting subsequent leaf appearance rates in these annual clovers.

3.5.3 Emergence and first leaf appearance

In all species, the emergence rate increased as the mean soil temperature increased (Figure 3.9), except for sowing dates on 26th February 2010, 30th March 2010 and 20th December 2010. Emergence was delayed on these sowing dates despite the application of irrigation, because of water stress in the field. This was caused by the use of an oscillating garden sprinkler which was inefficient in watering the sown plots as wind blew water into different directions. This caused less water to be applied on the sown plots. In addition, disruption of water supply occurred during the period of 20-22 December 2010 and 25-26 December 2010 due to an earthquake which damaged a hydrant pump in Iversen field, Lincoln University and caused disruption in irrigation activity. Moreover, in February, March and December 2010, evapotranspiration exceeded rainfall by four to five times (Figure 3.1), causing the soil surface to dry quickly and crust (Plate 2), which delayed seedling emergence.

Exclusion of these three sowing dates led to a constant thermal time requirement for emergence in ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers (Figure 3.9a) but not constant for ‘Bolta’ balansa or ‘Mihi’ Persian clovers (Figure 3.9b). During the period from late autumn to mid-spring sowing, all species took an average of 92 ^{o}C d to emerge above a T_b of 0^{o}C. As the temperature rose from late spring to summer, ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers maintained the same thermal time to emerge, but ‘Bolta’ balansa and ‘Mihi’ Persian clovers emerged faster at 37 (±12.2) ^{o}C d and 19 (±10.2) ^{o}C d respectively (Table 3.5). The delayed seedling emergence in February, March and December sowings had only a small affect on the timing of first leaf appearance (Figure 3.10b). However, it was assumed that the primordia of the first leaf was initiated at the same time as the cotyledons emerged which explained why the time to first leaf was largely unaffected and still conformed with the expected pattern. In support of this assumption, Dracup and Kirby (1996b) found that the primordia of the first and second leaves were initiated in the seed embryo of narrow-leafed lupin (*Lupinus angustifolius* L.) and they may also be present in these annual clovers.

3.5.4 Germination

In all species, the germination rate increased linearly with increased temperatures up to an optimum. Further increases in temperature then decreased the germination rate until no germination occured (Figure 3.6). The relationship between germination rate and temperature enabled the cardinal temperatures (base, optimum and maximum) to be defined. In this study, a two piece ‘broken stick’ regression model was appropriate for describing the germination response of annual clovers at the sub-optimal and supra-optimal range of temperatures. At the supra-optimal range, germination rate at 37.5 and 40 ^{o}C for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers deviated from the negative linear model (Figure 3.6) and were excluded from analysis. These were outside the species optimal thermal range (Angus *et al.*, 1981). Germination rate deviated because at high temperatures, seeds either germinated rapidly or died and germination rate was over estimated based on the germination duration of the surviving seeds. At 37.5 ^{o}C, the seeds of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers took six, three and five days respectively to germinate. However, the seed population that survived the 37.5^{o}C temperature were < 20% for both ‘Cefalu’ arrowleaf and

‘Prima’ gland clovers and < 45% for ‘Bolta’ balansa clover. Except for ‘Mihi’ Persian clover, no germination occured at 40 ^{o}C. The range of sub-optimal temperatures ($T \leq T_{opt}$) encompassed all of the soil temperatures experienced throughout a year in Iversen field, Lincoln University (Figure 3.8).

Within the range of sub-optimal temperatures, the final germination of each species was above 50%. The concept of thermal time summarized the range of individual temperature responses (Figure 3.3) into a single coefficient that can be used for all temperatures in the sub-optimal range. For example, the thermal time calculated for germination of ‘Cefalu’ arrowleaf clover was 30 ^{o}C d, hence at 5 ^{o}C ‘Cefalu’ arrowleaf could be expected to germinate in six days compared with two days at 15 ^{o}C. In all species, the T_b calculated were ≤ 4.5 ^{o}C and were not different from 0 ^{o}C (Table 3.4). These values of T_b were consistent with those reported by Monks (2009) and Lonati (2009) and therefore suggest that future work in annual clover species could assume a T_b of 0 ^{o}C for germination.

The use of 95% confidence interval to determine whether T_b was different from 0^{o}C (Table 3.4) highlights the need to understand the basis and limits of using linear relationships to describe development rate with temperature. This is because the linearity between development rate and temperature is only limited for a narrow range of temperatures. This relationship is practically exponential for temperatures approaching the T_b (Angus *et al.*, 1981; Bonhomme, 2000). In this study, the lowest temperature used for germination tests was 5 ^{o}C. Using lower temperatures (< 5^{o}C) enzymes are insufficiently flexible to carry the reaction; hence seeds either take too long to germinate or eventually rot and die. Given that the T_b is calculated from an extrapolation of the linear relationship between development rate and temperature, this method excludes the exponential relationship near the physiological T_b. Therefore, calculated T_b from extrapolation is often higher than the actual (physiological) T_b for which development is zero.

Non-linear models such as Lactin (Lactin *et al.*, 1995), Beta (Yan and Hunt, 1999), line plus exponential and critical exponential (standard curves from Genstat) were also tested to define cardinal temperatures for germination in this study but none of these models gave a better fit to the data points (based on the R^2 value) compared with the ‘broken-stick’ linear model. Therefore, the use of linear model was considered appropriate to describe development rate with temperature. It emphasizes the idea that T_b is only an approximation of the actual temperature threshold (Angus *et al.*, 1981; Bonhomme, 2000). Determining T_b is crucial to calculate thermal time accumulation between phenophases and when 95% confidence interval for T_b includes 0 ^{o}C, it is necessary to re-analyse thermal time using a T_b of 0 ^{o}C to allow comparisons among species (Moot *et al.*, 2000).

3.6 Conclusions

The experiments described in this chapter quantified the time for vegetative development in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers. Specific conclusions were:

1. Germination, emergence and first leaf appearance were quantified by thermal time. Phyllochron and axillary leaf appearance were quantified by thermal time when photoperiod at emergence was accounted for.

2. The thermal time requirements for germination were highest for ‘Cefalu’ arrowleaf (34 ^oC d) compared with ‘Bolta’ balansa (32 ^oC d), ‘Prima’ gland (28 ^oC d) and ‘Mihi’ Persian (25 ^oC d) clovers, above a T_b of 0^oC.

3. Thermal time to emergence was constant across all sowing dates for both ‘Cefalu’ arrowleaf (88 ^oC d) and ‘Prima’ gland (96 ^oC d) clovers above a T_b of 0^oC. ‘Bolta’ balansa and ‘Mihi’ Persian clovers took ~92 ^oC d (T_b = 0 ^oC) to emerge when sown during autumn to mid-spring but they emerged quicker during late spring to summer at 67 ^oC d (‘Bolta’ balansa) and 50 ^oC d (‘Mihi’ Persian), above a T_b of 0^oC.

4. ‘Cefalu’ arrowleaf clover took the longest time to produce its first leaf at 231 ^oC d compared with ‘Bolta’ balansa (212 ^oC d), ‘Prima’ gland (221 ^oC d) and ‘Mihi’ Persian (214 ^oC d) clovers, above a T_b of 0^oC.

5. In all species, the phyllochron was affected by photoperiod at emergence. The phyllochron decreased as the photoperiod increased (after 21 June up to 22 December) and rose as photoperiod shortened into early autumn, then declined again as photoperiod decreased into winter. Throughout all the sowing dates, phyllochron was fastest for ‘Prima’ gland (33 – 91 ^oC d) and slowest for ‘Cefalu’ arrowleaf (53 – 116 ^oC d) compared with ‘Bolta’ balansa (44 – 82 ^oC d) and ‘Mihi’ Persian (61 – 93 ^oC d) clovers.

6. In all species, the time to axillary leaf production differed with sowing dates. These differences were due to the differences in phyllochron at sowing rather than a difference

in the number of leaves initiated before secondary leaf production. Therefore, slower phyllochron resulted in longer time to the first axillary leaf but the leaf number in which it appeared was conservative.

4 Reproductive development

4.1 Introduction

In Chapter 3, vegetative development of four annual clovers was quantified. In this chapter, the drivers of reproductive development are considered. Together they quantify the duration of the annual life cycle of these clovers which can be used to facilitate on-farm decision making in relation to grazing management and seed set for regeneration.

The time to flowering was initially quantified using calendar days, followed by thermal time and photothermal time. The influence of photoperiod on the time of flowering was assessed at different seedling morphological stages (i.e. cotyledons, spade leaf and first trifoliate leaf appearance) to determine any photoperiod sensitivity phase. Time to flowering was then examined in relation to the changes in direction of photoperiod. In addition, the duration of absolute photoperiod when flowering occurred was quantified.

For each species, a visual reproductive development scale was created which could be used as a field guide to document the physical changes as the inflorescence progresses from bud visible to seed maturity. From these visual reproductive scales, the time from bud visible to full flower and duration of seed filling were quantified in thermal units.

Thus, the objectives were (1) to quantify the time to first flower across the 10 sowing dates, (2) to determine whether unifying relationships could be found for this and (3) to quantify the duration from flowering to physiological maturity.

4.2 Materials and methods

The experimental design was described in Section 3.2.2.2. Briefly, a split-plot factorial in a randomised complete block was sown with main plots as sowing date and sub-plots as species. Four replicates of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clover were sown on 10 dates (Table 3.3). Plots were sown as pure swards and managed without grazing or cutting to assess how seasonal changes in temperature and daylength affected development.

4.2.1 Measurements

4.2.1.1 Thermal time and photoperiod

Thermal time analysis used air temperature (1200 mm) and T_b of 0^oC (Section 3.2.2.5.4). Photoperiod was calculated as described in Section 3.2.2.5.2. Photoperiod at the time of emergence (cotyledons), first (spade) leaf and first trifoliate leaf appearance were tested against time to flowering to determine the photoperiod inductive phase. Based on the coefficient of determination (R^2) and biological interpretation sense (Figure 3.12), photoperiod at the time of first trifoliate leaf appearance was used.

4.2.1.2 Photothermal time

Photothermal time (Pt) was calculated using the following equations (Weir *et al.*, 1984) (Section 2.4.3):

Equation 4.1 $Pt = FP \times Tt \qquad 0 \leq FP \leq 1.0$

Equation 4.2 $FP = (Pp - Pp_{base})/(Pp_{opt} - Pp_{base})$

Where FP is a photoperiod modification factor, Pp is the photoperiod at first trifoliate leaf, Pp_{opt} is the optimum photoperiod and Pp_{base} is the base photoperiod. By multiplying FP with thermal time, the thermal time accumulation from emergence to flowering will be reduced. For Lincoln, Canterbury, New Zealand (43° 38'S, 172° 28'E), the Pp_{opt} was set to 16.7 hours and the Pp_{base} was set to 10.0 hours to correspond to the maximum and minimum photoperiods in the year.

4.2.1.3 Time to flowering

Development was inspected at 4 – 6 day intervals from 10 marked plants in each subplot starting from emergence. The first sign of reproductive development was after axillary leaf production. The marked plants were the same as those used for tracking vegetative development (Section 3.2.2.5.4). The final number of main stem leaves at flowering was also recorded. The time of flowering was defined as when the first bud was visible in the axil of leaves on 50% of the marked plants. Peak flowering was defined when ≥ 50% of the plant population within a plot had fully open flowers (Plates 6-9).

Plate 7 Peak flowering in ‘Cefalu’ arrowleaf clover.

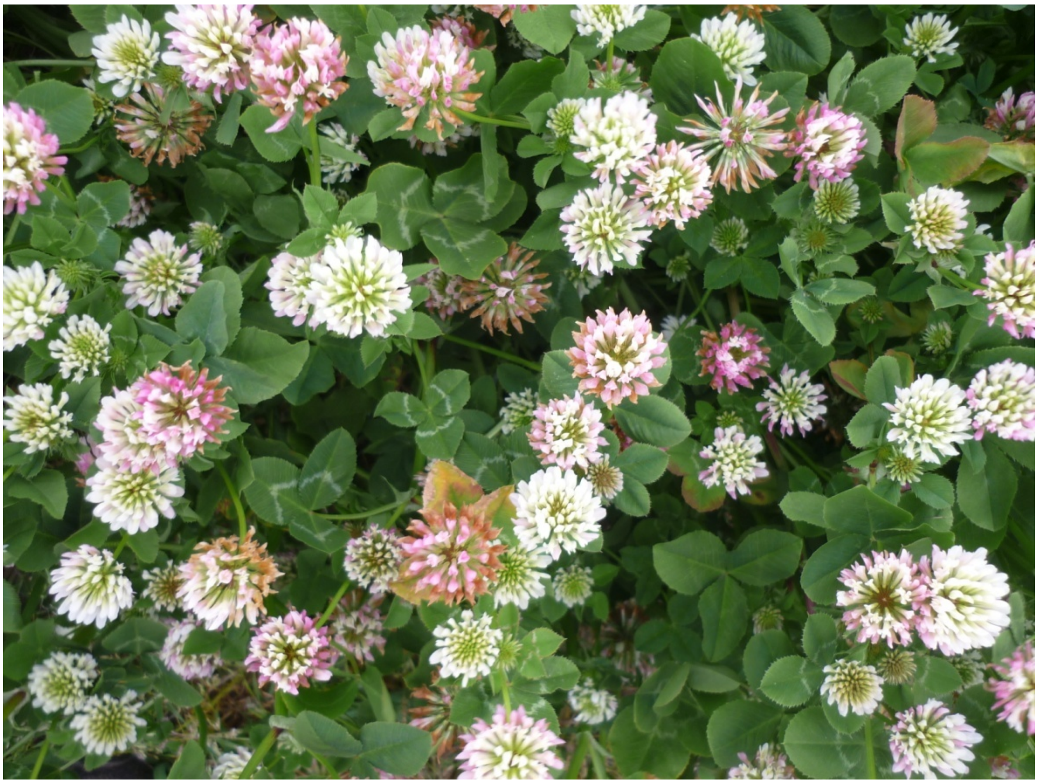


Plate 8 Peak flowering in ‘Bolta’ balansa clover.

Plate 9 Peak flowering in ‘Prima’ gland clover.

Plate 10 Peak flowering in ‘Mihi’ Persian clover.

4.2.1.4 Reproductive development

4.2.1.4.1 From bud visible to open flower

The first three inflorescences per marked plant were tagged as they appeared and their reproductive structures were recorded at 4-6 day intervals from bud visible to open flower using the visual reproductive development scale (Section 4.2.1.4.3).

4.2.1.4.2 From peak flowering to seed maturity

An open flower indicates that the inflorescence is available for pollination. Pollination was recorded as soon as the basal inflorescence turned brown (for arrowleaf, balansa and Persian clover) or purple (for gland clover). As pollination occured, 100 inflorescences per subplot were marked with a Max 'Tapener' HT-B2, fixed around the peduncle for detailed observation. The time of pollination was chosen for inflorescence marking because it is the starting point of seed formation. It also allows comparison with previous work (Monks, 2009) with balansa clover. For each species, starting on the day of marking, five inflorescences were harvested every four days. These were dissected and observed in detail using numeric scores from the visual reproductive development scale (Section 4.2.1.4.3). The inflorescences were then dried in a force-draught oven at 60^{o}C for 48 hours and weighed. Harvesting and recording stopped when 100% of seeds had turned red/brown (stage 13 for arrowleaf clover) and the seed pods had shattered (stage 16 for balansa, stage 13 for gland and stage 12 for Persian clover).

4.2.1.4.3 Reproductive development scale

The visual reproductive development scale was generated with a numeric score that represented the progress of the inflorescence to harvest maturity in arrowleaf, balansa, gland and Persian clovers. The reproductive development scale for balansa clover was generated by Monks (2009) over three seasons (2005-2007) and was used to track development of balansa clover inflorescences in this study. The scales developed for arrowleaf, gland and Persian clover inflorescence were based on this with modification to account for the different reproductive structures for each species.

Table 4.1 Visual scale (numeric) outlining the reproductive development of an arrowleaf clover inflorescence

---

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 florets within the inflorescence have a visible corolla

5 Full flower – 100% of corolla have the standard unfolded from the wings

6 <50% of the inflorescence turned brown as an indication of pollination[1]

7 >50% of the inflorescence are brown. Pods are formed within the inflorescence starting from the basal inflorescence

8 50% of pods are formed in the inflorescence

9 >90% of the inflorescence is brown. > 50% of pods are formed

10 Seeds in the bottom pod turned yellow

11 Seeds in the bottom pod turned red/brown. 50% of seeds turned yellow.

12 50% of seeds turned red/brown

13 100% of seeds turned red/brown (5 YR 5/10, 2.5 YR 3/8, 10 R 3/2)

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

[1] Petals that are brown/wilted with age are not counted.

Illustration is shown in Plate 11.

(a)

(b)

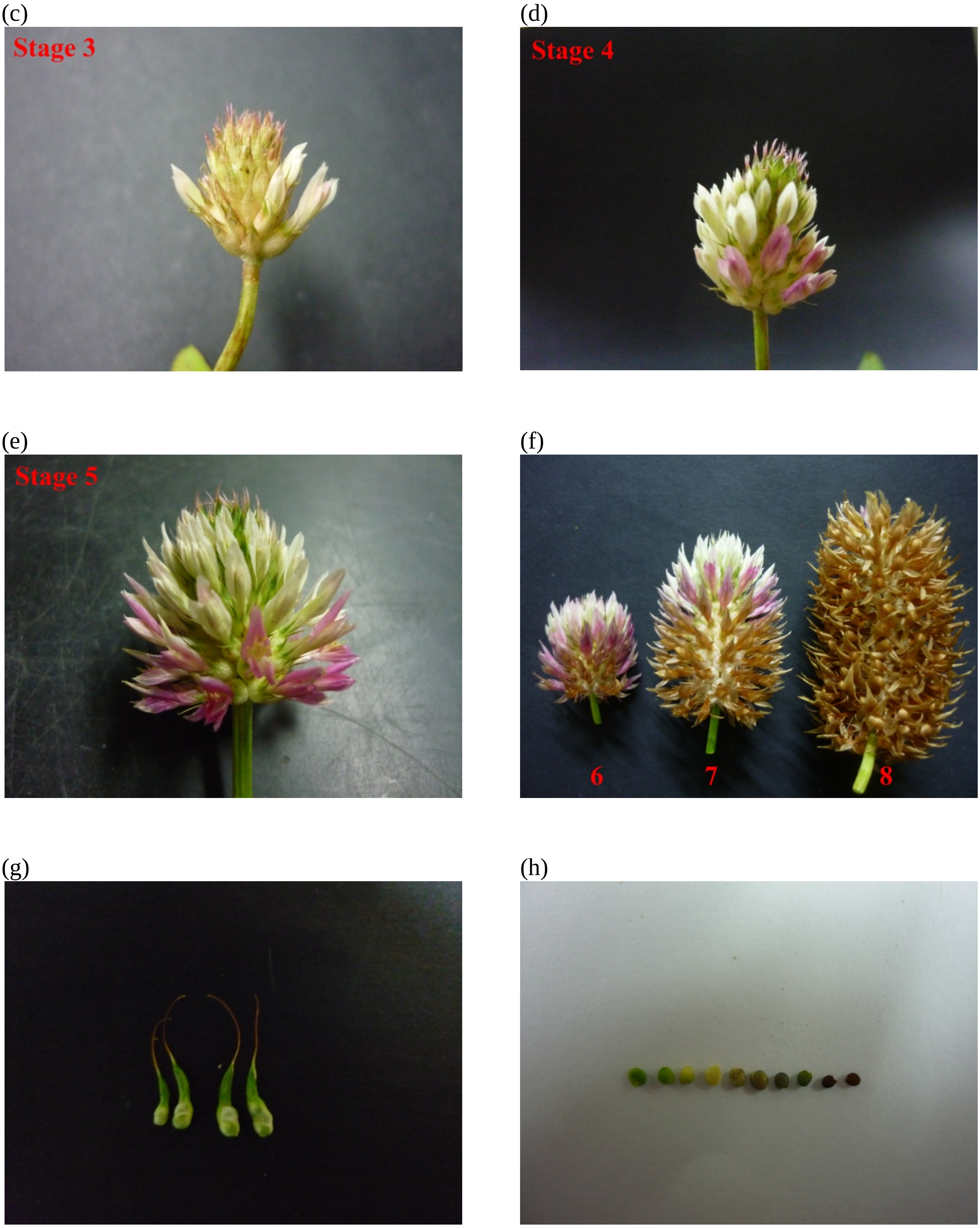


Plate 11 Illustration of reproductive development showing (a) stage 1 (b) stage 2 (c) stage 3 (d) stage 4 (e) stage 5 (f) stage 6,7,8 (g) pod filling (h) seed development in ‘Cefalu’ arrowleaf clover.

Table 4.2 Visual scale (numeric) outlining the reproductive development of a balansa clover inflorescence (From Monks, 2009)

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 florets within the inflorescence have a visible corolla

5 Full flower – 100% of corolla have the standard unfolded from the wings

6 All florets within inflorescence show browning as an indication of pollination[1]

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 (2.5Y (8/8 to 10) or 5Y (8/8 to 10)

13 100% of pods are yellow

14 First sign of seeds darkening (7.5YR (6/8) to 5 YR (2/3))

15 100% of seeds are dark (7.5 YR (6/8) to 5 YR (2/3))

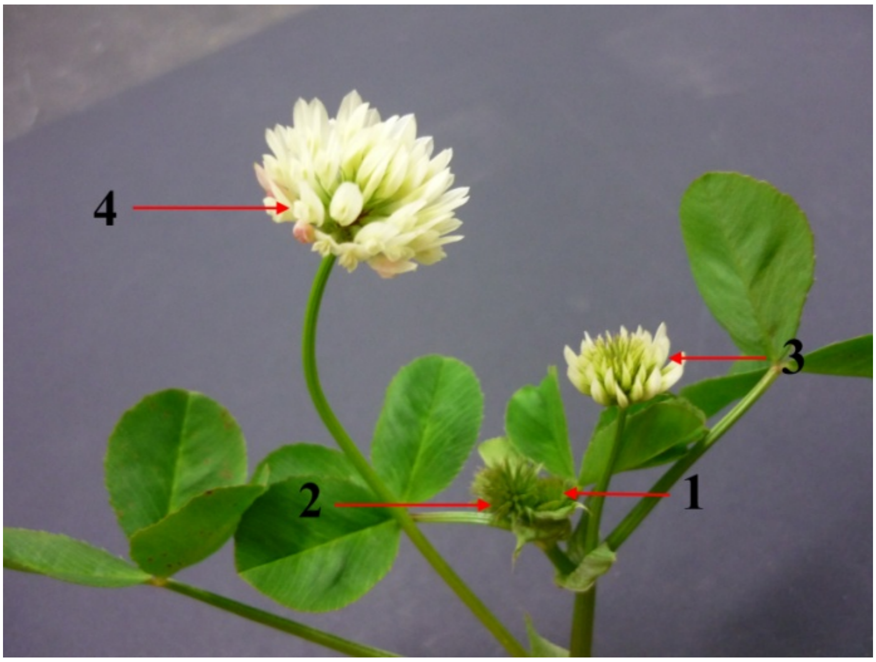
16 Seed shatter upon burst pods

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

[1] Petals that are brown/wilted with age are not counted.

Illustration is shown in Plate 12.

(a)



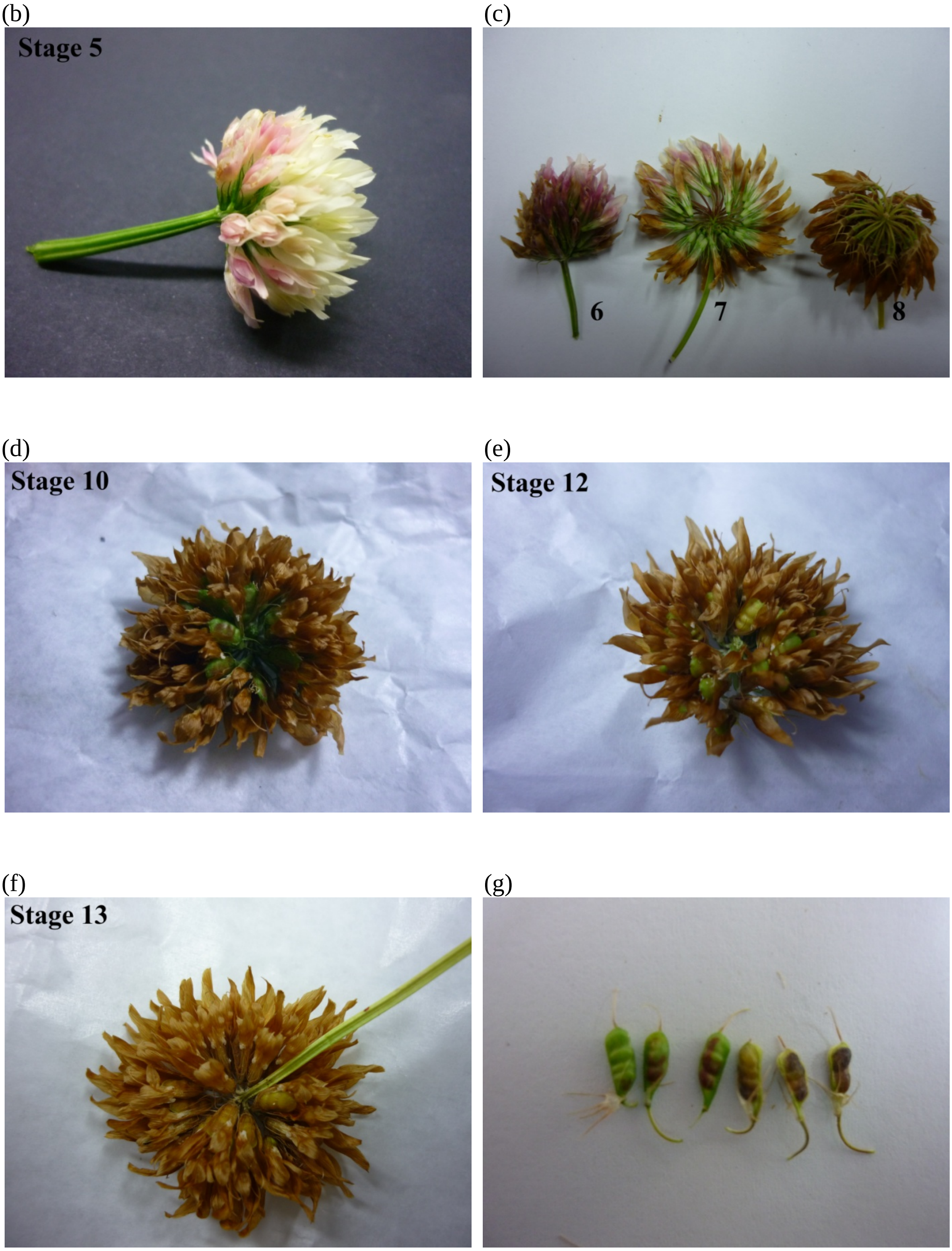


Plate 12 Illustration of reproductive development showing (a) stage 1,2,3,4 (b) stage 5 (c) stage 6,7,8 (d) stage 10 (e) stage 12 (f) stage 13 (g) pod development in ‘Bolta’ balansa clover.

Table 4.3 Visual scale (numeric) outlining the reproductive development of a gland clover inflorescence

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 florets within the inflorescence have a visible corolla

5 Full flower – 100% of corolla have the standard unfolded from the wings

6 Florets on the base inflorescence turned purple as an indication of pollination[1]

7 Florets on the top inflorescence turned purple. Abscission layer formed starting from the base inflorescence

8 Pods are visible within the inflorescence

9 Pods enlarge, green (G or GY) in colour

10 Formation of one or more complete seed. Seeds are green in colour (5 GY (5/10 to 6/8))

11 Pods are green yellow (2.5 GY (8/4 to 8/6)). First sign of seed yellowing (5 Y 8/4 to 2.5 GY 8/10)

12 Seeds are hard and 100% of seeds are yellow (2.5 Y 8/6 to 5 Y 8/8)

13 Seeds shatter upon rubbing

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

[1] Petals that are purple/wilted with age are not counted

Illustration is shown in Plate 13.

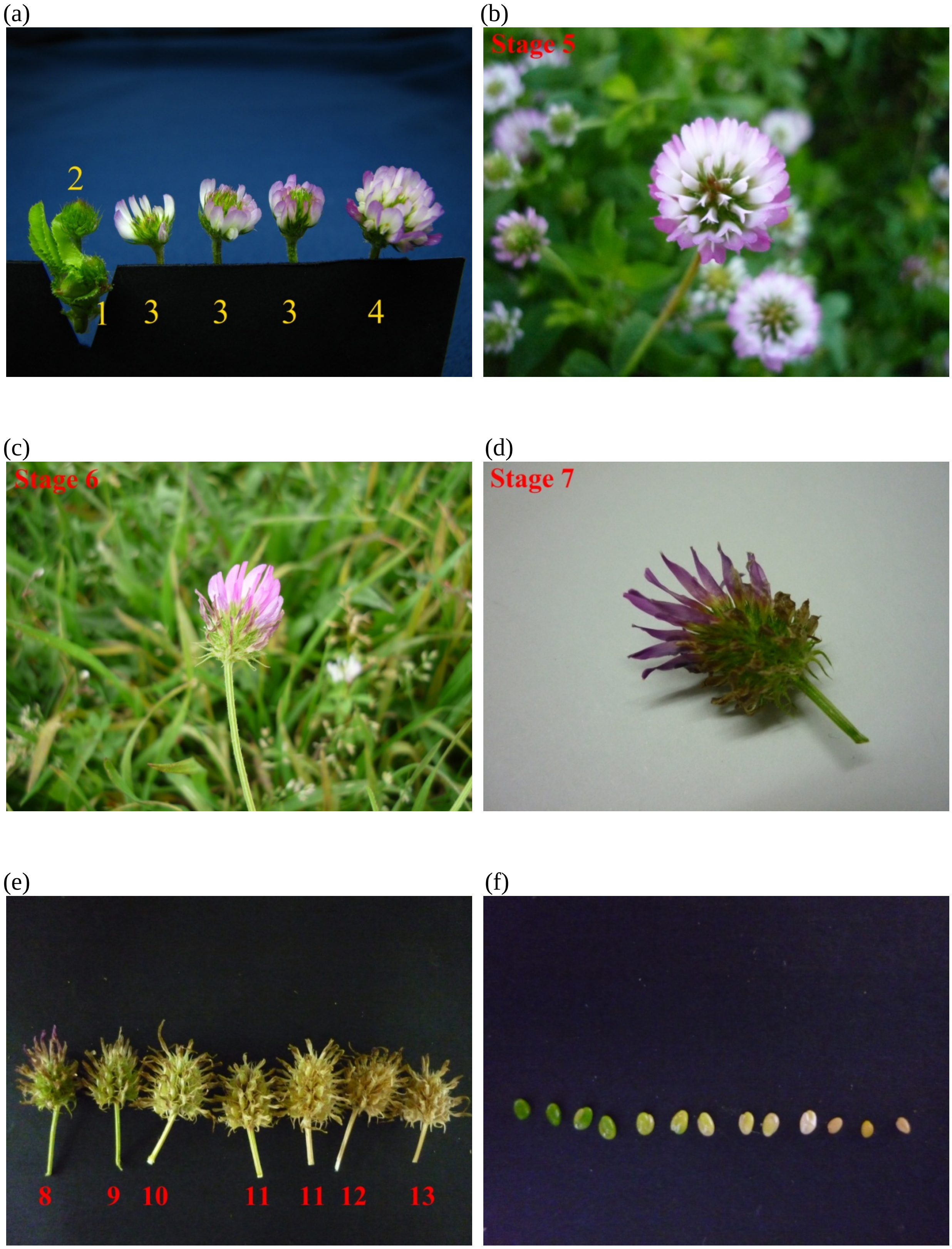


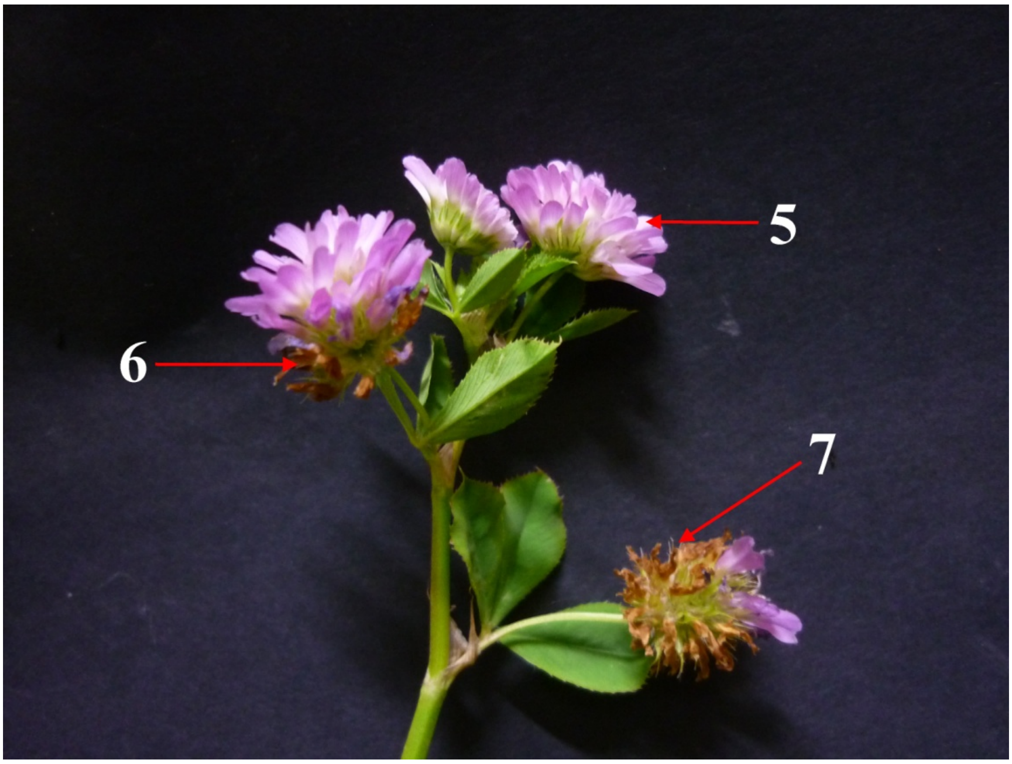
Plate 13 Illustration of reproductive development showing (a) stage 1,2,3,4 (b) stage 5 (c) stage 6 (d) stage 7 (e) stage 8,9,10,11,12,13 (f) seed development in ‘Prima’ gland clover.

Table 4.4 Visual scale (numeric) outlining the reproductive development of a Persian clover inflorescence

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 florets within the inflorescence have a visible corolla
5	Full flower – 100% of corolla have the standard unfolded from the wings
6	Florets turned brown as an indicator of pollination starting from the basal inflorescence[1]
7	All florets within the inflorescence turned brown
8	Inflorescence swell, pods start to form within the inflorescence
9	Pods enlarge, green in colour
10	Pods turned yellow. Seeds are green in colour
11	Pods turned brown. First sign of seeds change colour
12	Pods burst

Note: Values within parentheses correspond to Munsell colour charts for plant tissues.
[1] Petals that are brown/wilted with age are not counted
Illustration is shown in Plate 14.

(a)



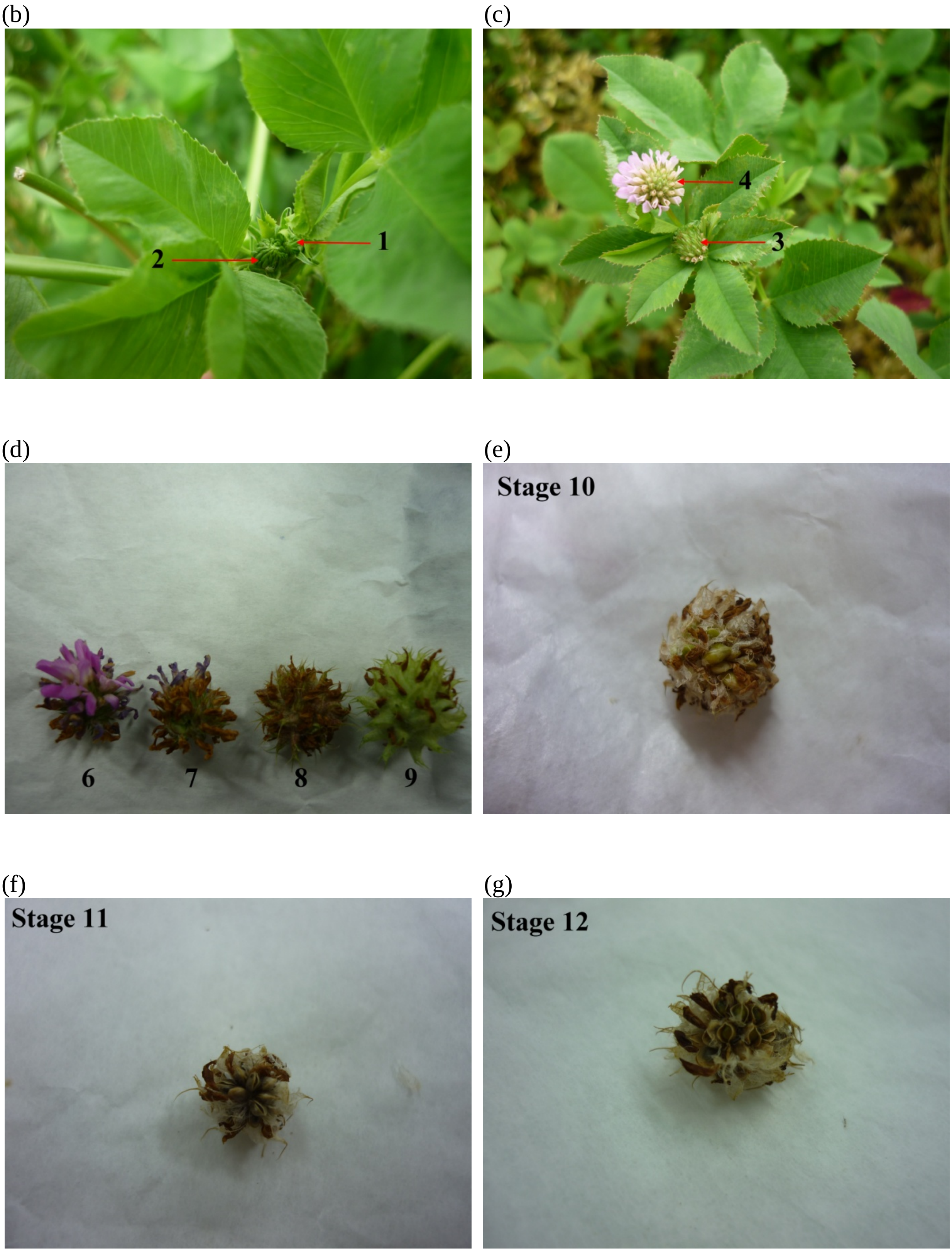


Plate 14 Illustration of reproductive development showing (a) stage 5,6,7 (b) stage 1,2 (c) stage 3,4 (d) stage 6,7,8,9 (e) stage 10 (f) stage 11 (g) stage 12 in 'Mihi' Persian clover.

4.3 Data analysis

The time from emergence to flowering was analysed using calendar days, thermal time and photothermal time. Thermal time and photothermal time to flowering were also analyzed in relation to photoperiod at seedling emergence (cotyledon), first (spade) leaf and first trifoliate leaf appearance (Section 4.2.1.1). The time from first bud visible to open flower, and from pollination to seed maturity was determined using both calendar days and thermal time. The weight of inflorescence was plotted against thermal time from pollination to determine physiological maturity of the seed. Physiological maturity was defined as the time of maximum dry weight of the inflorescence, based on the assumption that seed filling was completed at this point (Hyde, 1950).

4.4 Results

4.4.1 Calendar days from emergence to flowering

The number of days from emergence to flowering (Stage 1) in ‘Cefalu’ arrowleaf and ‘Bolta’ balansa clovers decreased with each successive sowing date from 26th February 2010 to 20th December 2010 but then increased for the 19th January 2011 sowing date (Figure 4.1). For ‘Prima’ gland clover, time to flowering was shorter for plants sown on 26th February 2010 (126 (±7.2) days) compared with 30th March 2010 (147 (±0.8) days). However, the number of days to flowering showed a decreasing trend from late March to December sowings. For ‘Mihi’ Persian clover, the number of days to flowering declined from 253 (±1.6) when sown on 26th February 2010 to 74 (±1.3) when sown on 25th September 2010. Sowing on 9th November resulted in a slightly longer time (86 (±2.7) days) to flower. Flowering did not occur for ‘Mihi’ Persian clover sown on 20th December 2010 or 19th January 2011. The plants sown on these dates died in August 2011, with no visible signs of reproductive development. There was not a constant number of days to flowering for all the species. To account for these variations, time to flowering was quantified in thermal time.

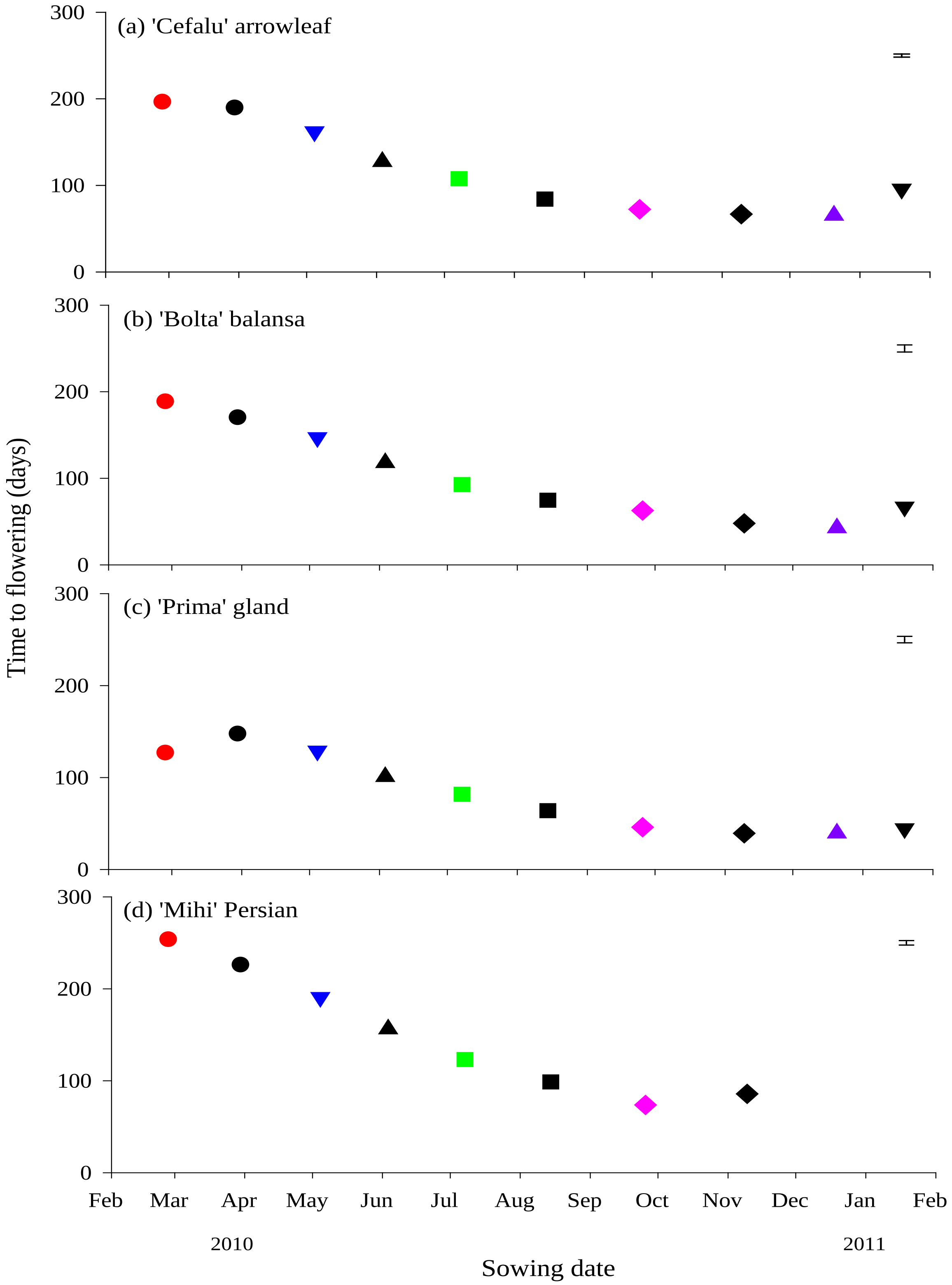


Figure 4.1 Number of days from emergence to flowering for (a) 'Cefalu arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d)'Mihi' Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). See Table 3.3 for actual sowing date (SD). Error bars represent the maximum standard error of the mean.

4.4.2 Thermal time from emergence to flowering

Thermal time was quantified to summarize the duration from emergence to flowering (Stage 1, first bud visible).

4.4.2.1 ‘Cefalu’ arrowleaf clover

When crops were sown throughout autumn and winter, thermal time to flowering decreased systematically from 1694 (±17.7) ^{o}C d (after appearance of 14 nodes) for 30 March sowing to 940 (± 7.0) ^{o}C d (10 nodes) for 14 August sowing (Figure 4.2a, Figure 4.3a). For spring sowings, the crops took an average of 958 (±21.2) ^{o}C d to flower. Crops sown on 20 December took 965 (±52.3) ^{o}C d (14 nodes) to flower but this increased to 1834 (±47.3) ^{o}C d (16 nodes) for those sown on 26 February.

4.4.2.2 ‘Bolta’ balansa clover

The thermal time requirement for flowering in ‘Bolta’ balansa clover also declined with each successive sowing date from 1733 (±25.3) ^{o}C d (27 nodes) for those sown on 26 February to 597 (±18.5) ^{o}C d (11 nodes) for those sown on 20 December (Figure 4.2b,Figure 4.3b). However, sowing ‘Bolta’ balansa clover on 19 January delayed flowering to 918 (±50.9) ^{o}C d after 18 nodes had been produced on the main stem.

4.4.2.3 ‘Prima’ gland clover

The thermal time to flowering increased as summer progressed for gland clover starting from a minimum of 520 (±11.1) ^{o}C d (13 nodes) for those sown on 20 December to 1216 (±9.2) ^{o}C d (13 nodes) for those sown on 30 March (Figure 4.2c, Figure 4.3c). It then declined gradually from 977 (±22.6) ^{o}C d (11 nodes) for 4 May sowing back to 503 (±7.7) ^{o}C d (11 nodes) for 9 November sowing date. Hence, sowing in late spring resulted in the lowest thermal time accumulation to flower for ‘Prima’ gland clover.

4.4.2.4 ‘Mihi’ Persian clover

‘Mihi’ Persian clover took the longest time to flower at 2610 (±28.6) ^{o}C d (31 nodes) when sown on 26 February (Figure 4.2d, Figure 4.3d). The time to flowering then shorten with sequential sowing dates until 25 September (1047 (±23.9) ^{o}C d, after 14 nodes). Sowing ‘Mihi’ Persian clovers on 9 November delayed flowering until 1468 (±51.9) ^{o}C d (19 nodes).

Furthermore sowing on 20 December and 19 January, resulted in no flowering and the plants died in the following winter.

Crops that flowered later produced the highest number of nodes before initiation of the first flower. The minimum number of nodes to flowering was 10 for both ‘Cefalu’ arrowleaf and ‘Bolta’ balansa, 9 for ‘Prima’ gland and 12 for ‘Mihi’ Persian clover and was associated with early flower initiation (Figure 4.3). The difference in thermal time to flowering with sowing dates suggests the time to flowering was not solely controlled by thermal time accumulation. To account for this, the effect of photoperiod on flowering was also investigated.

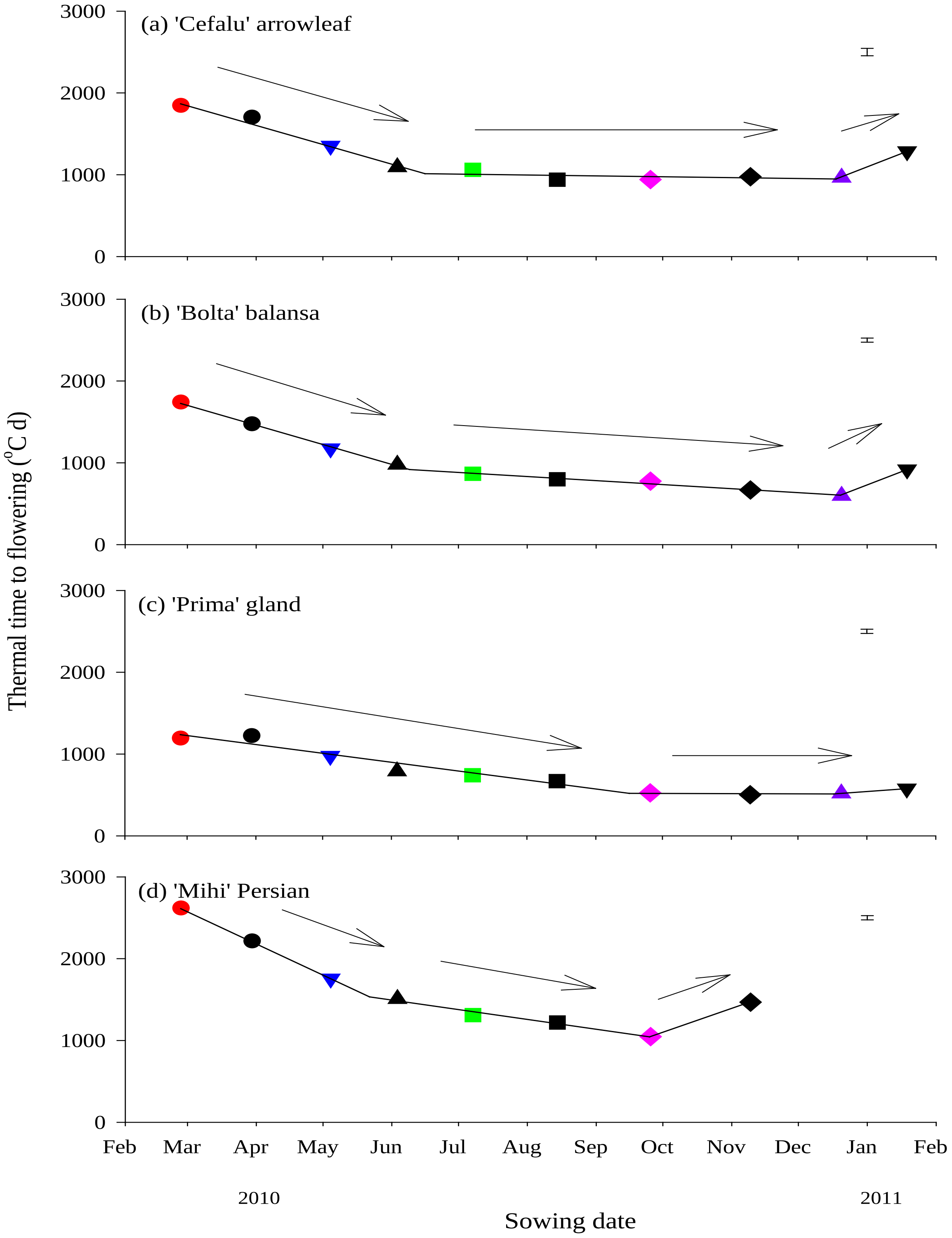


Figure 4.2 Thermal time from emergence to flowering for a) ‘Cefalu arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d)‘Mihi’ Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). Arrows indicate decreasing and increasing thermal time across SD. Error bars represent the maximum standard error of the mean. Note: Thermal time used air temperature (T_b = 0°C). See Table 3.3 for actual sowing date (SD).

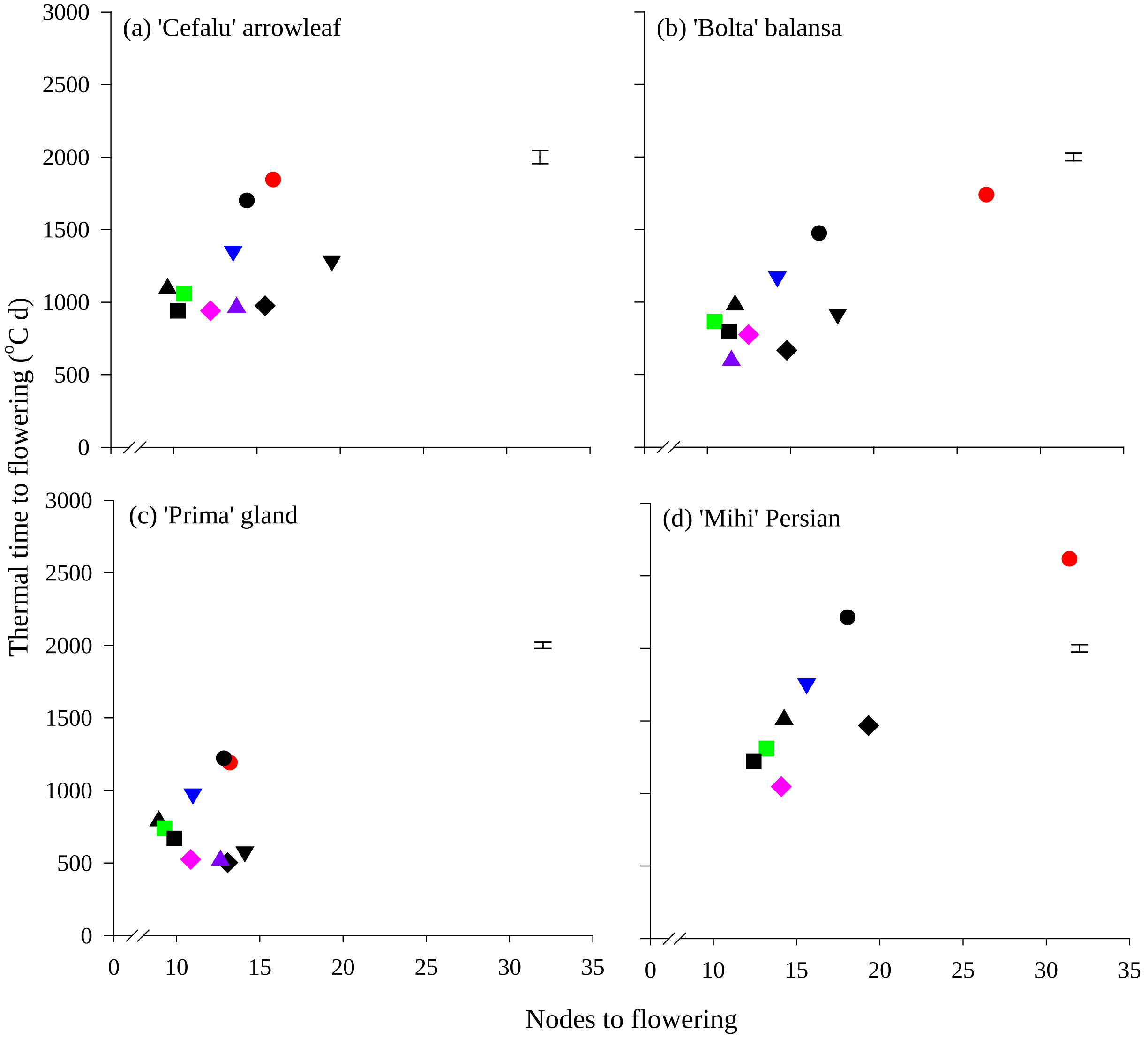


Figure 4.3 Thermal time to flowering against number of nodes to the first flower for (a) ‘Cefalu arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d)‘Mihi’ Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). Error bars represent the maximum standard error of the mean. Note: Thermal time used air temperature ($T_b = 0^{o}C$). See Table 3.3 for actual sowing date (SD).

4.4.3 Thermal time to flowering in relation to photoperiod

For each species, the thermal time required for flowering exhibited a hysteresis with changes (hours) and directions (increase and decrease) of photoperiod at first trifoliate (second) leaf stage (Figure 4.4). When seedlings produced their first trifoliate leaf following the shortest day (21 June) into an increasing photoperiod (up to 16.3 hours for 'Cefalu' arrowleaf, 'Bolta' balansa and 'Prima' gland and 14.6 hours for 'Mihi' Persian clover), the duration to flowering decreased by 21 (±9.6) ^{o}C d hour^{-1} for 'Cefalu' arrowleaf, 55 (±6.9) ^{o}C d hour^{-1} for 'Bolta' balansa, 50 (±6.7) ^{o}C d hour^{-1} for 'Prima' gland and 115 (±11.7) ^{o}C d hour^{-1} for 'Mihi' Persian clover (Table 4.5). Following the longest day (21 December), as photoperiod shortened to ~13 hours into the autumn, time to flowering rose rapidly by 254 (±22.5) ^{o}C d hour^{-1} for 'Cefalu' arrowleaf, 337 (±4.5) ^{o}C d hour^{-1} for 'Bolta' balansa and 205 (±39.1) ^{o}C d hour^{-1} for 'Prima' gland clover. However, as photoperiod continued to decrease further into the winter, the thermal time requirement for flowering decreased by 162 (±50.6) ^{o}C d hour^{-1} for 'Cefalu' arrowleaf, 183 (±29.3) ^{o}C d hour^{-1} for 'Bolta' balansa, 209 ^{o}C d hour^{-1} for 'Prima' gland and 277 (±23.8) ^{o}C d hour^{-1} for 'Mihi' Persian clover.

The minimum absolute photoperiod when flowering commenced was 11.3 hours for 'Cefalu' arrowleaf, 12.6 hours for 'Bolta' balansa, 10.3 hours for 'Prima' gland and 15.4 hours for 'Mihi' Persian clover.

Table 4.5 Coefficients of fitted relationship between thermal time to flowering (^{o}C d) and direction of photoperiod (hours) change at first trifoliate leaf stage in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	$y = 1285 - 20.9x$	43
	Decrease into autumn	$y = 5138 - 253.5x$	98
	Decrease into winter	$y = -231 + 162.4x$	82
'Bolta' balansa	Increase	$y = 1525 - 54.6x$	93
	Decrease into autumn	$y = 6146 - 336.8x$	100
	Decrease into winter	$y = -645 + 183.1x$	95
'Prima' gland	Increase	$y = 1309 - 50.1x$	92
	Decrease into autumn	$y = 3841 - 205.1x$	93
	Decrease into winter	$y = -1128 + 209.4x$	
'Mihi' Persian	Increase	$y = 2713 - 114.6x$	97
	Decrease into winter	$y = -1012 + 277.4x$	99

R^2, coefficient of determination.

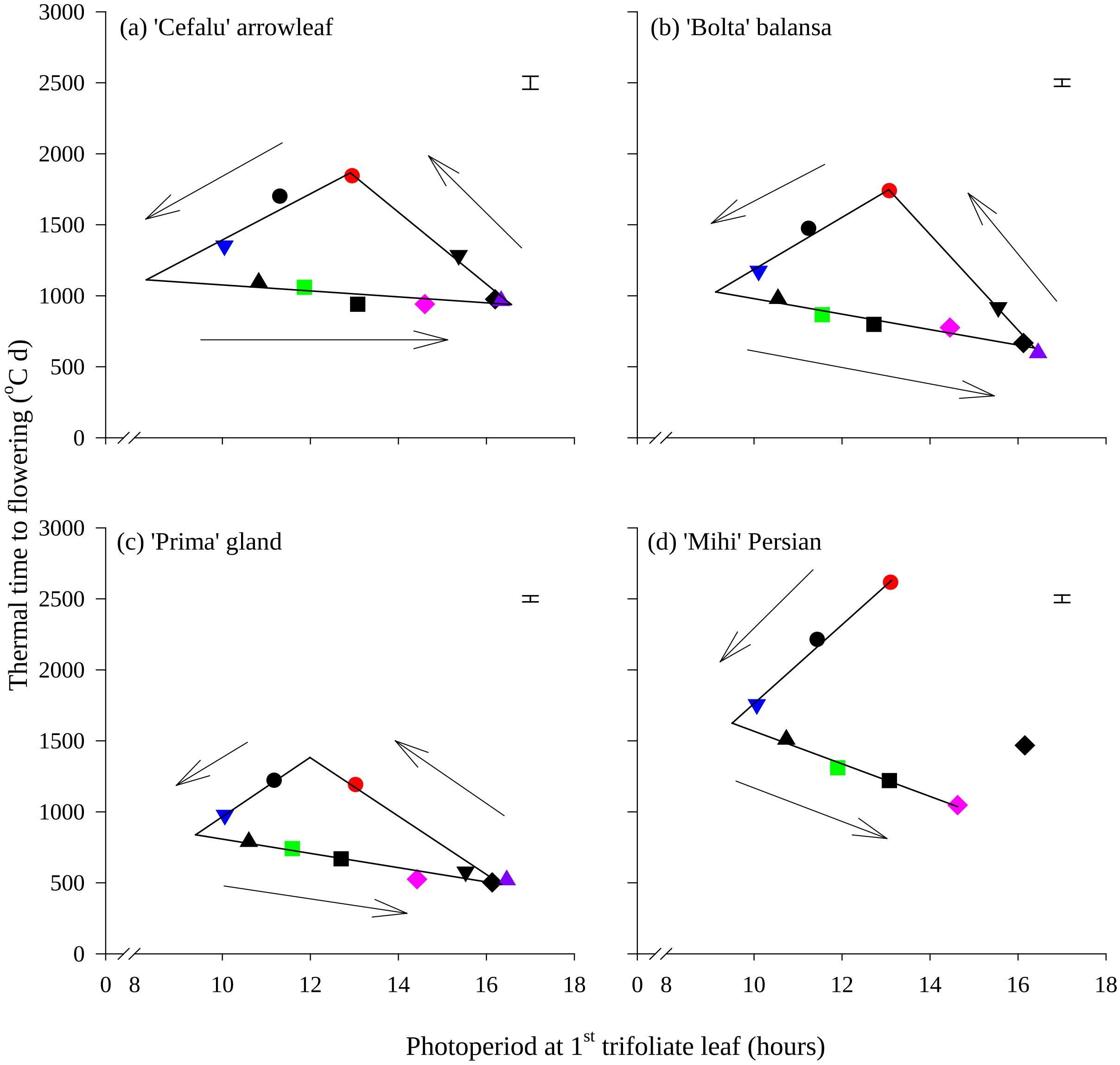


Figure 4.4 Thermal time to flowering in response to photoperiod of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). See Table 3.3 for actual sowing date (SD). Arrows indicate direction towards increase and decrease photoperiod. Error bars represent the maximum standard error of the mean. Note: Thermal time used air temperature ($T_b = 0^oC$).

4.4.4 Photothermal time to flowering

The accumulation of thermal time from emergence to flowering was adjusted by incorporating photoperiod effective hours to calculate photothermal time (Equation 4.1). This modification reduced the accumulation of thermal time. In all species, photothermal time to flowering declined between the 26th February 2010 and 4th May 2010 sowings and increased between 3rd June and 19th January 2011 (Figure 4.5). The maximum photohermal time to flowering was reduced to 1038 (±81.6) °C d for 'Cefalu' arrowleaf, 804 (±17.7) °C d for 'Bolta' balansa, 541 (±20.1) °C d for 'Prima' gland and 1359 (±50.5) for 'Mihi' Persian clover.

Photothermal time to flowering still produced a hysteresis with changes in direction of photoperiod at emergence (Figure 4.6). In all species, photothermal time to flowering declined with decreasing photoperiod and increased with increasing photoperiod. The relationships between photothermal time and direction of photoperiod are summarised in Table 4.6.

Table 4.6 Coefficients of fitted relationship between photothermal time to flowering (°C d) and direction of photoperiod (hours) change at first trifoliate leaf stage in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	$y = -1431 + 143.6x$	100
	Decrease into winter	$y = -2804 + 278.6x$	100
'Bolta' balansa	Increase	$y = -888 + 92.9x$	97
	Decrease into autumn	$y = 1560 - 56.1x$	32
	Decrease into winter	$y = -2691 + 266.0x$	99
'Prima' gland	Increase	$y = -696 + 73.0x$	98
	Decrease into autumn	$y = 712 - 13.5x$	20
	Decrease into winter	$y = -1790 + 178.8x$	100
'Mihi' Persian	Increase	$y = -2029 + 200.8x$	94
	Decrease into winter	$y = -4014 + 397.0x$	99

R^2, coefficient of determination.

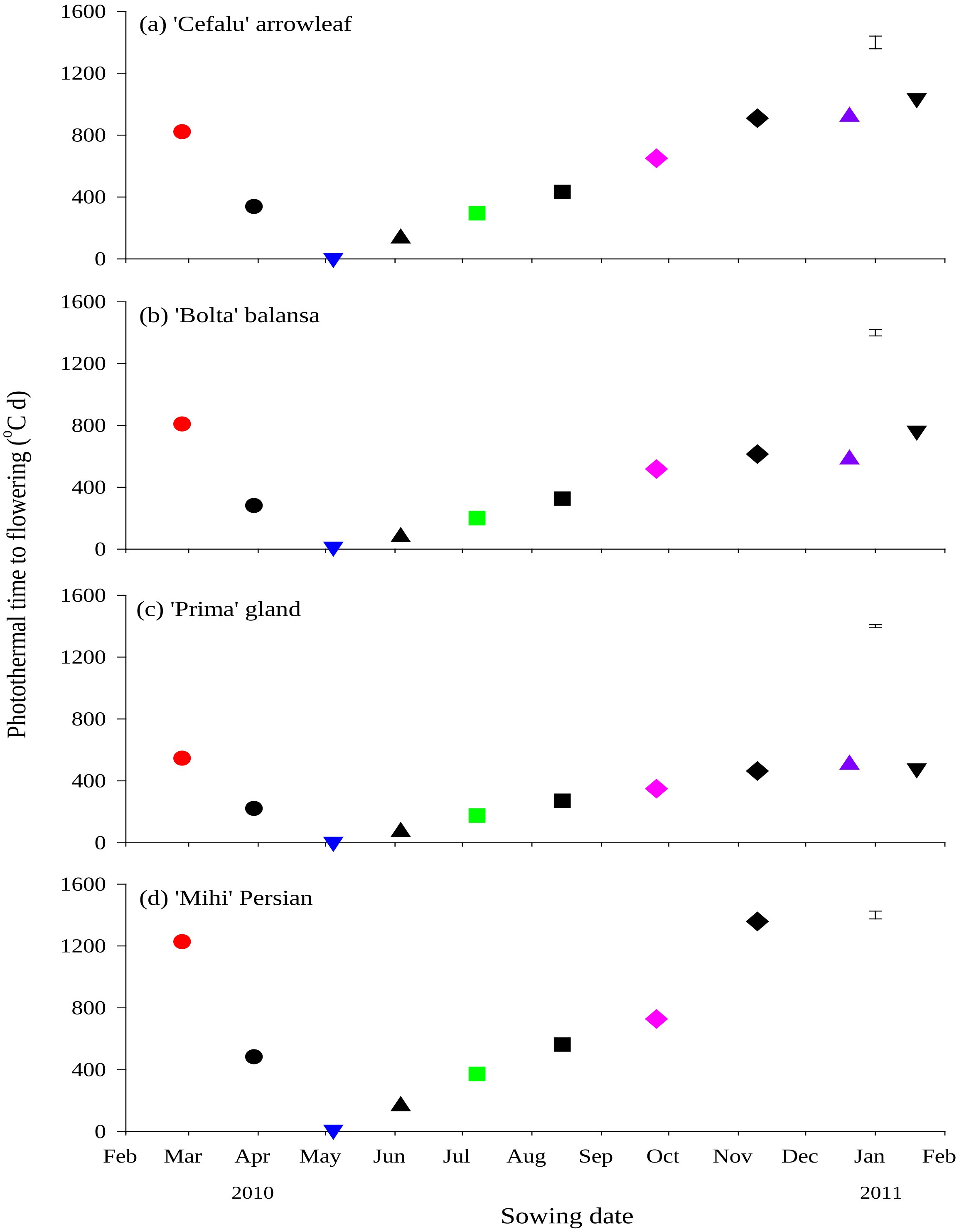


Figure 4.5 Photothermal time from emergence to flowering for a) ‘Cefalu arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d)‘Mihi’ Persian clovers sown on 10 dates at Lincoln University, Canterbury, New Zealand. SD1(🔴), SD2(●), SD3(▼), SD4(▲), SD5(🟩), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). See Table 3.3 for actual sowing date (SD). Error bars represent the maximum standard error of the mean. Note: Thermal time analysis used air temperature (T_b = 0°C).

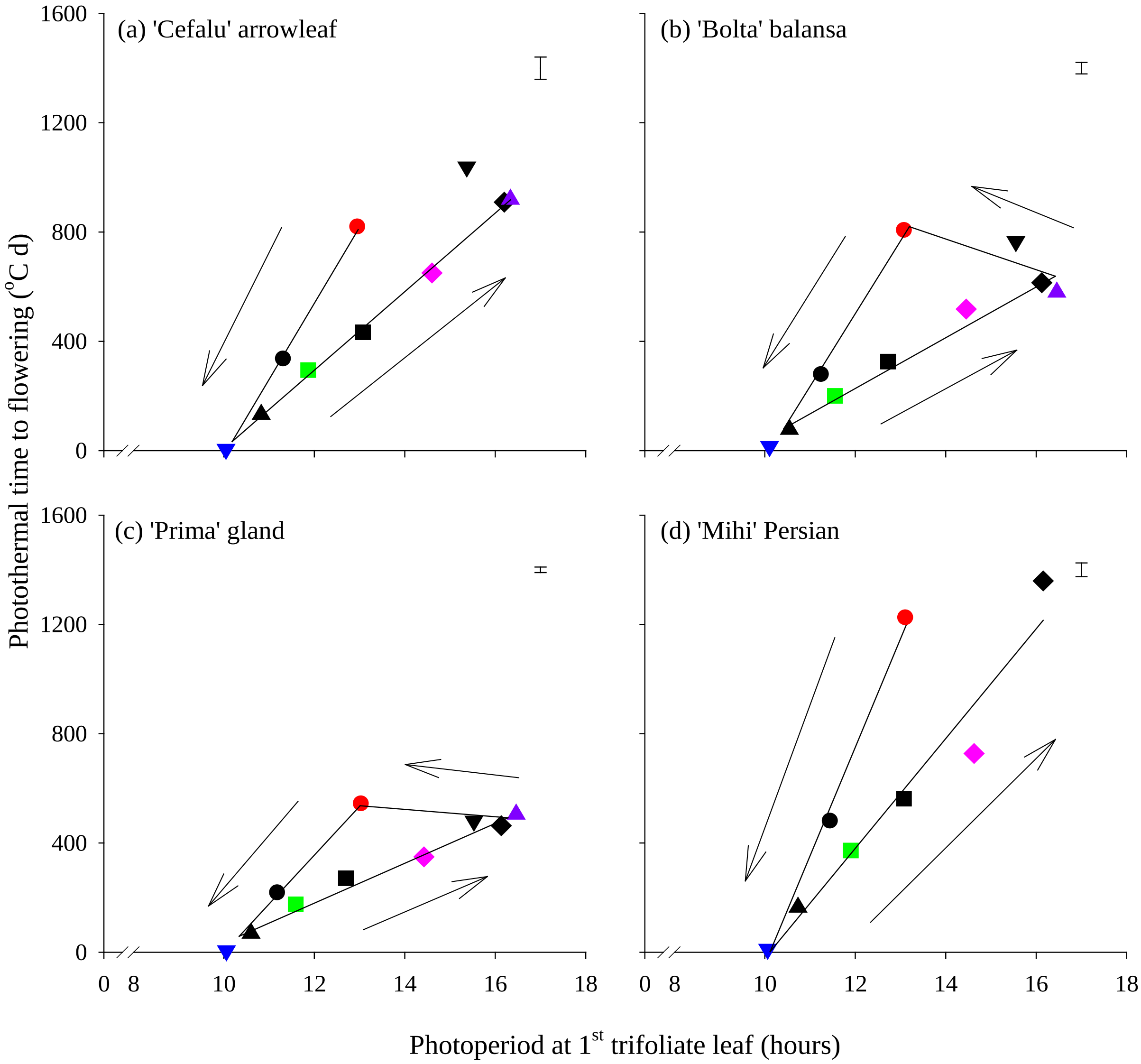


Figure 4.6 Photothermal time to flowering in response to photoperiod at emergence of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown on 10 dates in Lincoln University, Canterbury, New Zealand. SD1(●), SD2(●), SD3(▼), SD4(▲), SD5(■), SD6(■), SD7(◆), SD8(◆), SD9(▲), SD10(▼). Arrows indicate direction towards increase and decrease photoperiod. Error bars represent the maximum standard error of the mean. Note: Thermal time analysis used air temperature (T_b = 0°C). For actual sowing dates (SD), see Table 3.3.

4.4.5 Reproductive development scale

4.4.5.1 The duration from bud visible to open flower

The thermal time accumulation from bud visible (stage 1) to open flower (stage 5) averaged 341 (±7.0) ^{o}C d for ‘Cefalu’ arrowleaf, 215 (±4.5) ^{o}C d for ‘Bolta’ balansa, 196 (±5.2) ^{o}C d for ‘Prima’ gland and 186 (±6.4) ^{o}C d for ‘Mihi’ Persian clovers across all sowing dates (Table 4.7).

‘Cefalu’ arrowleaf clover sown on 19th January 2011 did not produce open flowers. The plants had their bud visible in late autumn (29th April 2011) but these did not develop any further and died in mid winter.

Table 4.7 Thermal time accumulation (^{o}C d) from bud visible (stage 1) to open flower (stage 5) in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on 10 different dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

Sowing date	Species			
(SD)	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26/2/2010	341	237	214	210
30/3/2010	369	215	219	204
4/5/2010	366	230	216	208
3/6/2010	354	227	198	180
7/7/2010	326	237	218	172
14/8/2010	304	197	184	163
25/9/2010	303	190	181	167
9/11/2010	327	206	167	182
20/12/2010	378	192	175	-
19/1/2011	-	222	185	-
Mean	341	215	196	186
Max. s.e.	31.9	22.0	31.5	28.2
P-value	0.148	0.059	0.175	0.430

Thermal time quantified based on air temperature (T_b = 0^{o}C). Max. s.e., maximum standard error of the mean. Date of flowering is shown in Appendix 6.

4.4.5.2 Inflorescence weight as an indicator of seed maturity

4.4.5.2.1 Maximum weight of inflorescence

Except for ‘Cefalu’ arrowleaf clover, the maximum weight of inflorescence in the other species differed ($P<0.05$) with sowing date (Table 4.8). The maximum weight of inflorescence in ‘Cefalu’ arrowleaf clover averaged 0.74 (±0.029) g across all sowing dates. For ‘Bolta’ balansa clover, the weight of inflorescence declined with successive sowing dates from 0.28 (±0.017) g for plants that flowered in early spring (SD1) to 0.13 (±0.006) g for those that flowered in mid-summer (SD9) (Figure 4.7b). The maximum weight of inflorescence in ‘Prima’ gland clover ranged from 0.10 (±0.001) to 0.13 (±0.005) g. For ‘Mihi’ Persian clover, the maximum weight of inflorescence was constant at an average of 0.11 (±0.006) g for those that flowered in late spring (between SD1 and SD4) but started to decrease with seasonal changes down to 0.07 (±0.007) g in late summer (SD8). The maximum dry weight of inflorescences (physiological maturity) coincided with the following visual reproductive stage in each clover species:

Clover species:	Visual reproductive stage:	
‘Cefalu’ arrowleaf	(12)	50% of seeds turned red/brown (Table 4.1)
‘Bolta’ balansa	(13)	100% of pods are yellow (Table 4.2)
‘Prima’ gland	(12)	Seeds are hard and 100% of seeds are yellow (Table 4.3)
‘Mihi’ Persian	(11)	Pods turned brown and first sign of seeds change colour (Table 4.4)

Table 4.8 Inflorescence maximum dry weight (g) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on 10 different dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

Sowing date	Species			
(SD)	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26/2/2010	0.73	0.28	0.10	0.11
30/3/2010	0.83	0.26	0.12	0.12
4/5/2010	0.94	0.27	0.12	0.11
3/6/2010	0.71	0.21	0.13	0.11
7/7/2010	0.70	0.20	0.12	0.10
14/8/2010	0.67	0.18	0.10	0.08
25/9/2010	0.74	0.19	0.10	0.08
9/11/2010	0.64	0.14	0.11	0.07
20/12/2010	-	0.13	0.10	-
19/1/2011	-	-	-	-
S.E.M.	0.029	0.009	0.003	0.004
Max. s.e.	0.155	0.020	0.015	0.019
P-value	0.168	<.001	0.017	0.008
L.S.D. (5%)	-	0.036	0.019	0.030

S.E.M. standard error of the mean. Max. s.e., maximum standard error of the mean. L.S.D., Least significant differences.

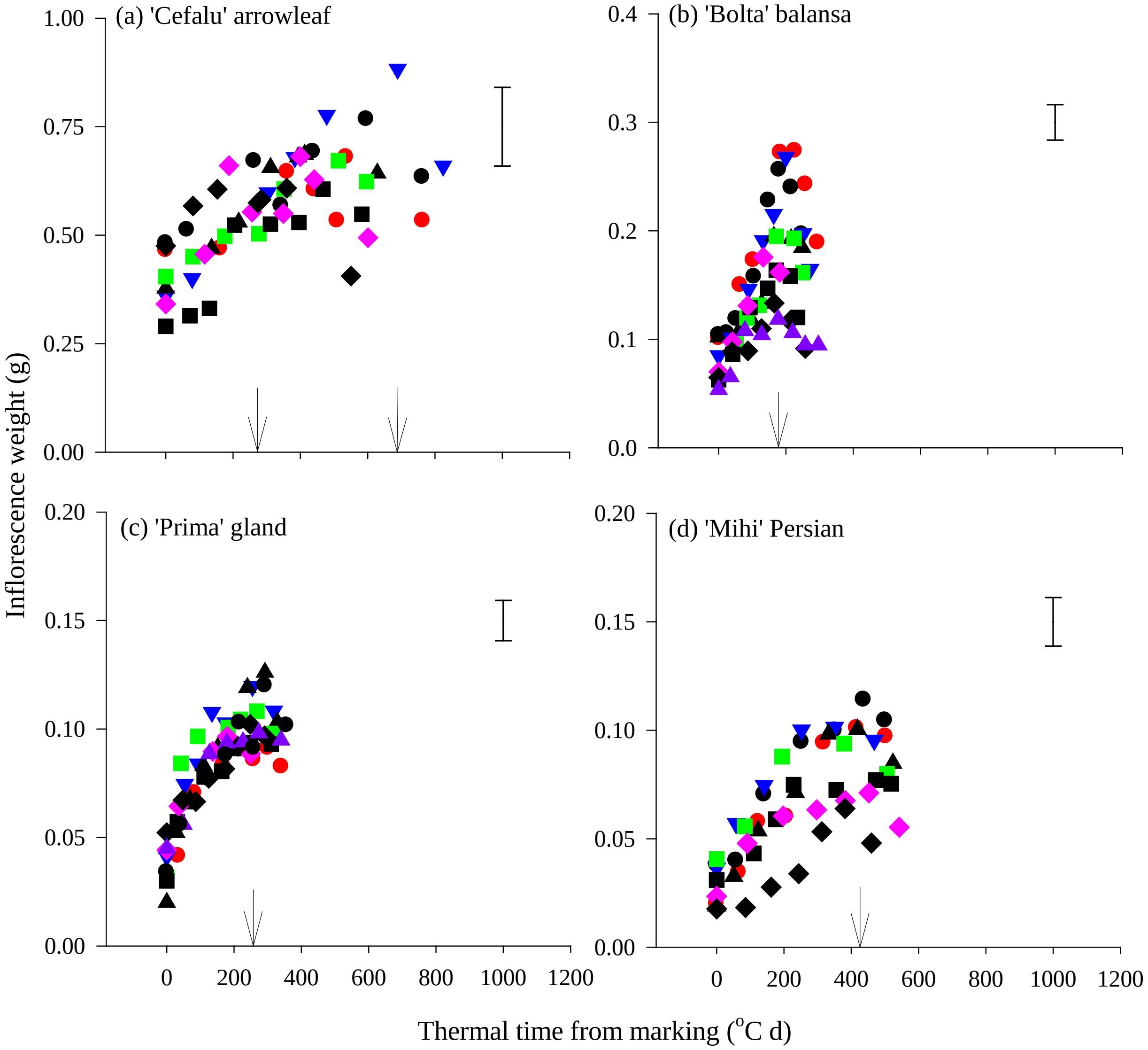


Figure 4.7 The inflorescence weight of (a) ‘Cefalu’ arrowleaf (b) ‘Bolta’ balansa (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers against thermal time from pollination (stage 6) sown at different dates in Lincoln University, Canterbury, New Zealand. SD1(🔴), SD2(●), SD3(▼), SD4(▲), SD5(🟩), SD6(■), SD7(◆), SD8(◆), SD9(▲). Arrows indicate physiological maturity. Error bars represent the maximum standard error of the mean. Note: Thermal time analysis used air temperature (T_b = 0^{o}C for arrowleaf and Persian, T_b = 5.2^{o}C for balansa and T_b = 4.3^{o}C for gland clover). For actual sowing dates (SD), see Table 3.3.

4.4.5.2.2 Thermal time from pollination (stage 6) to physiological maturity

Thermal time accumulation from pollination (stage 6) to physiological maturity (maximum seed yield) for 'Cefalu' arrowleaf clover differed ($P<0.05$) with sowing dates (Table 4.9). It ranged from 274 (±96.4) ^{o}C d in SD8 to 689 (±44.2) ^{o}C d in SD3 plants (Figure 4.7a). The duration of seed filling was constant for 'Bolta' balansa (average of 185 (±15.8) ^{o}C d), 'Prima' gland (average of 256 (±26.8) ^{o}C d) and 'Mihi' Persian clover (average of 425 (±19.8) ^{o}C d) across all sowing dates. For 'Cefalu' arrowleaf clover, the duration of seed filling was linearly (R^2 = 74%) related to the maximum weight of the inflorescence (Figure 4.8).

Table 4.9 Thermal time accumulation (^{o}C d) from pollination (stage 6) to physiological maturity in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on 10 different dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

Sowing date	Species			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26/2/2010	509	227	265	491
30/3/2010	596	172	310	404
4/5/2010	689	197	226	401
3/6/2010	393	177	279	383
7/7/2010	513	209	239	502
14/8/2010	467	177	264	473
25/9/2010	400	157	193	415
9/11/2010	274	188	242	330
20/12/2010	-	163	291	-
19/1/2011	-	-	-	-
Mean	480	185	256	425
S.E.M.	29.7	15.8	26.8	19.8
Max. s.e.	96.4	30.1	36.9	73.7
P-value	<.001	0.084	0.135	0.103
L.S.D. (5%)	101.7	-	-	-

Thermal time quantified based on air temperature ($T_b = 0^{o}$C for arrowleaf and Persian, $T_b = 5.2^{o}$C for balansa and $T_b = 4.3^{o}$C for gland clover). SD, Sowing date. S.E.M. standard error of the mean. Max. s.e., maximum standard error of the mean. L.S.D., Least significant differences. Date of pollination is shown in Appendix 7.

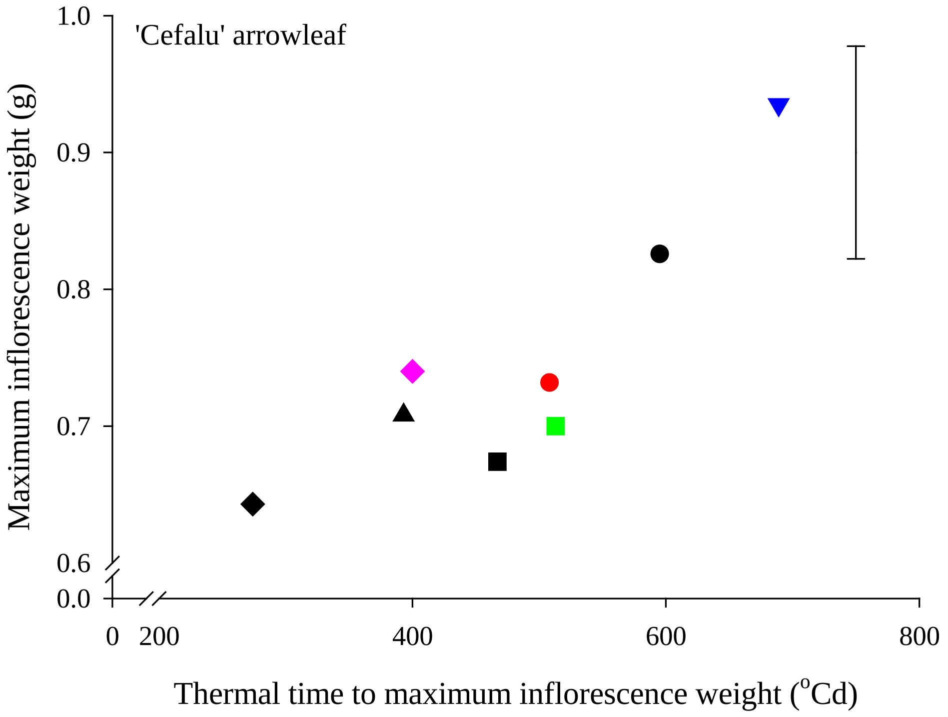


Figure 4.8 The maximum inflorescence weight of ‘Cefalu’ arrowleaf clover against thermal time accumulation from pollination (stage 6) to maximum inflorescence weight sown on eight different dates at Lincoln University, Canterbury, New Zealand. SD1 (🔴), SD2 (●), SD3 (▼), SD4 (▲), SD5 (🟩), SD6 (■), SD7 (◆), SD8 (◆). Error bars represent the maximum standard error of the mean. Note: Thermal time analysis used air temperature (T_b = 0°C). For actual sowing dates (SD), see Table 3.3.

4.5 Discussion

4.5.1 Time of flowering

The time to flowering was measured in calendar days, thermal time and photothermal time. Both days and thermal time to flowering showed a systematic variation across sowing dates and therefore, the influence of photoperiod on flowering was investigated.

4.5.2 Thermal time to flowering in relation to photoperiod

The thermal time to flowering showed a hysteresis based on the perception of photoperiod at seedling emergence (Figure 4.4). In all species, autumn sown plants that emerged into a declining photoperiod took the longest time to produce their first floral bud. As daylength continued to decrease further into winter, the thermal time requirement to flowering decreased systematically with subsequent sowing dates. In contrast, all plants that emerged after the shortest day (21 June) into an increasing photoperiod only required about half of the thermal time accumulation to flower as of autumn sown crops. These results show a strong influence of photoperiod on the time of flower initiation in these annual clovers.

For ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers, their time to flowering was shortest with increasing photoperiods which indicates that these species are facultative long day plants. A similar result was found by Monks *et al*., (2010) on the flowering pattern of ‘Bolta’ balansa clover. In contrast, ‘Cefalu’ arrowleaf clover had a constant time to flower throughout the lengthening photoperiod (Figure 4.4a) which showed a characteristic of an obligate long day plant, where flowering occurs when a specific duration of photoperiod is exceeded. Evans *et al*., (1992) reported that subterranean clovers are facultative long day plants and their time to flowering shortened as sowing was delayed from late autumn to late spring in Australia. In the Mediterranean region (latitude range 30-50 ^{o}N), where these annual clover species originate, flowering is enhanced as daylength exceeds their critical photoperiod (Del Pozo and Aronson, 2000), which confirms all four species as a long day plants.

Time to flowering was delayed as photoperiod shortened after the longest day (21 December) into autumn. This hysteresis, with the increasing and decreasing of photoperiod, showed that these annual clovers responded to the photoperiod ‘clock’. Photoperiod sensitivity has been reported to begin at the time of seedling emergence (when the coleoptile was visible) in wheat (*Triticum* spp.) crops (Brooking *et al.*, 1995; Hay and Porter, 2006). However, this may not be applicable to legume crops. In annual clovers, emergence was defined when cotyledons were visible. Time to emergence was shown to be constant across sowing dates and was unaffected by photoperiod (Figure 3.9). The first leaf produced was a spade leaf and is not a true leaf. The time to first (spade) leaf was also constant with sowing and not controlled by photoperiod (Figure 3.10). The first true leaf produced was a trifoliate (second) leaf and the leaf appearance rate or phyllochron was shown to be affected by the photoperiod (Figure 3.12). The relationship between time to flowering and photoperiod was tested at emergence (Appendix 4), spade leaf (Appendix 5) and first trifoliate leaf stage (Table 4.5). The strongest relationship was found when tested against photoperiod at first trifoliate leaf stage. Therefore, it is believed that these annual clovers had a short juvenile or non inductive phase before photoperiod sensitivity commenced at the time of first trifoliate leaf appearance.

At this time, the plant has determined which primordia in the shoot apex would turn into the first flower or which would continue to become a leaf depending on their response to daylength (Brooking *et al.,* 1995; Evans, 1959; Hay and Delecolle, 1989; Miglietta, 1991). Hence, plants that emerged in autumn, into the shortening photoperiod, produced more leaves and extended their vegetative growth more than those that emerged in spring into a lengthening photoperiod. For example, 'Mihi' Persian clover sown on 26 February produced 32 nodes to flowering but those sown on 16 August only required 12 nodes to flowering (Figure 4.3d). This response serves as a survival strategy. By producing more leaves, autumn sown plants can avoid flowering in unfavourable conditions during winter. They will flower only shortly earlier than spring sown crops when photoperiod increases. In addition, increasing photoperiods coincide with increasing temperature and decreasing moisture, so flowering at this time would give favourable conditions for pollinating insects and promote reproductive development.

In all species, flowering occurred when the absolute photoperiod was between 11.3-16.6 hours for 'Cefalu' arrowleaf, 12.6-16.6 hours for 'Bolta' balansa, 10.3-16.6 hours for 'Prima' gland and 15.4-16.6 hours for 'Mihi' Persian clover. Thus, photoperiod effect delayed flowering until early spring for both 'Cefalu' arrowleaf and 'Bolta' balansa, late winter for 'Prima' gland and late spring for 'Mihi' Persian clover. Particularly in 'Mihi'Persian clover, it only flowered between mid-November and mid-December (Appendix 6). The requirement of specific photoperiod for Persian clover to flower might be related to its centre of origin

(Section 2.3.4). In these locations, photoperiod on the longest day of the year is about 16 hours and this may act as environmental signal for flower initiation. This could explain the delay in flowering behaviour of ‘Mihi’ Persian clover.

4.5.3 Photothermal time to flowering

The use of photothermal time was expected to unify the time to flowering by multiplying the accumulated thermal time from emergence to flowering with a photoperiod modification factor (value 0-1). The aim was to remove the hysteresis of flowering time with photoperiod and to describe the flowering response in a single relationship. However, the use of photothermal time to quantify time to flowering failed to account for the differences in flowering time between plants that emerged into the same absolute photoperiod but into a different direction (increasing/decreasing) of photoperiod. For example, plants sown on 26 February 2010 and 25 September 2010 emerged at a 13.6 hour photoperiod but into decreasing and increasing photoperiods, respectively. The differences in flowering time were 497 ^{o}C d for ‘Cefalu’ arrowleaf, 521 ^{o}C d for ‘Bolta’ balansa, 348 ^{o}C d for ‘Prima’ gland and 907 ^{o}C d for ‘Mihi’ Persian clover (Figure 4.6).

Despite having adjusted thermal time with a photoperiod factor, time to flowering still exhibited a hysteresis with photoperiod. Thus, flowering response was unable to be unified using a single relationship. This implies that time of flowering is dependent on the direction of photoperiod at seedling emergence and not solely on the duration of photoperiod and thermal time. Monks *et al.*, (2010) reported the same results with ‘Bolta’ balansa clover. In contrast, Evans *et al.*, (1992) used a linear photothermal time model (Summerfield *et al.*, 1991) to predict time of flowering in subterranean clovers. Despite the strong relationship of flowering response to temperature and photoperiod, the photothermal models were least effective in prediction of flowering time for plants that emerged into decreasing photoperiod after autumn sowing, where time to flowering deviated from the predicted model. Therefore, quantifying the thermal time requirement at different photoperiods was considered the most suitable approach to quantify time to flowering and account for photoperiod (Figure 4.4).

4.5.4 Reproductive development

The rate of plant development after the first sign of flowering (i.e. first bud visible) was mainly driven by temperature. The estimated duration from bud visible to physiological maturity was 610-1025 ^{o}C d for 'Cefalu' arrowleaf, 400 ^{o}C d for 'Bolta' balansa, 452 ^{o}C d for 'Prima' gland and 611 ^{o}C d for 'Mihi' Persian clover. 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers had a determinate growth habit. The accumulation of thermal time from pollination to the maximum inflorescence weight (physiological maturity) for these three clover species was constant (Table 4.9). For 'Bolta' balansa and 'Prima' gland clovers, thermal time requirement for development after open flower (stage 5) suggests the use of a T_b above 5.2 ^{o}C and 4.3 ^{o}C, respectively (Figure 4.10). Thus, thermal time calculation from germination to open flower used T_b of 0^{o}C and then 5.2 ^{o}C (balansa clover) and 4.3^{o}C (gland clover) until physiological maturity. Similarly, Porter and Gawith (1999) calculated thermal time for development in wheat using a T_b of 1^{o}C until anthesis and then 9^{o}C until crop maturity. For 'Cefalu' arowleaf and 'Mihi' Persian clovers, thermal time requirement for all development was calculated using a T_b of 0 ^{o}C.

The duration of seed filling in 'Cefalu' arrowleaf clover differed among sowing dates. In 'Cefalu' arrowleaf, the individual inflorescence appeared to have the capacity to increase the number of florets as it elongated during the seed filling period (Plate 11f). This slowed the overall process of seed filling because the inflorescence kept producing new florets at the top for as long as the conditions were favourable, which suggests it has an indeterminate growth habit. This extended duration of seed filling, combined with favourable temperatures resulted in a heavier seed size and heavier weight per inflorescence. This was particularly true for plants (SD2 and SD3) that experienced seed filling during late spring to early summer period (Figure 4.8). In contrast, plants that flowered during late summer (SD8) had insufficient time to expand their inflorescence further due to warmer temperature that accelerated their maturity. Thus seed filling happened at the fastest rate plus their seed size was reduced.

In all species, seed weight was heaviest when seed filling occurred during mid-spring to early summer (Table 4.8) and this was associated with autumn sown plants. For spring sown plants that experienced seed filling during summer, the warmer ambient temperature which coincided with summer moisture deficits accelerated plant senescence. This shortened the duration of seed filling (Figure 4.9) and photosynthesis, thus reducing plant canopy expansion and photosynthate supply, eventually resulting in lower seed weight. The effects of warmer temperatures and drought on the duration of seed filling and seed size have been reported for spring wheat (*Triticum aestivum* L.) (Amir and Sinclair, 1991), faba bean (*Vicia faba* L.) (Mwanamwenge *et al.*, 1999) and soybean (*Glycine max* (L.) Merr.) (De Souza *et al.*, 1997).

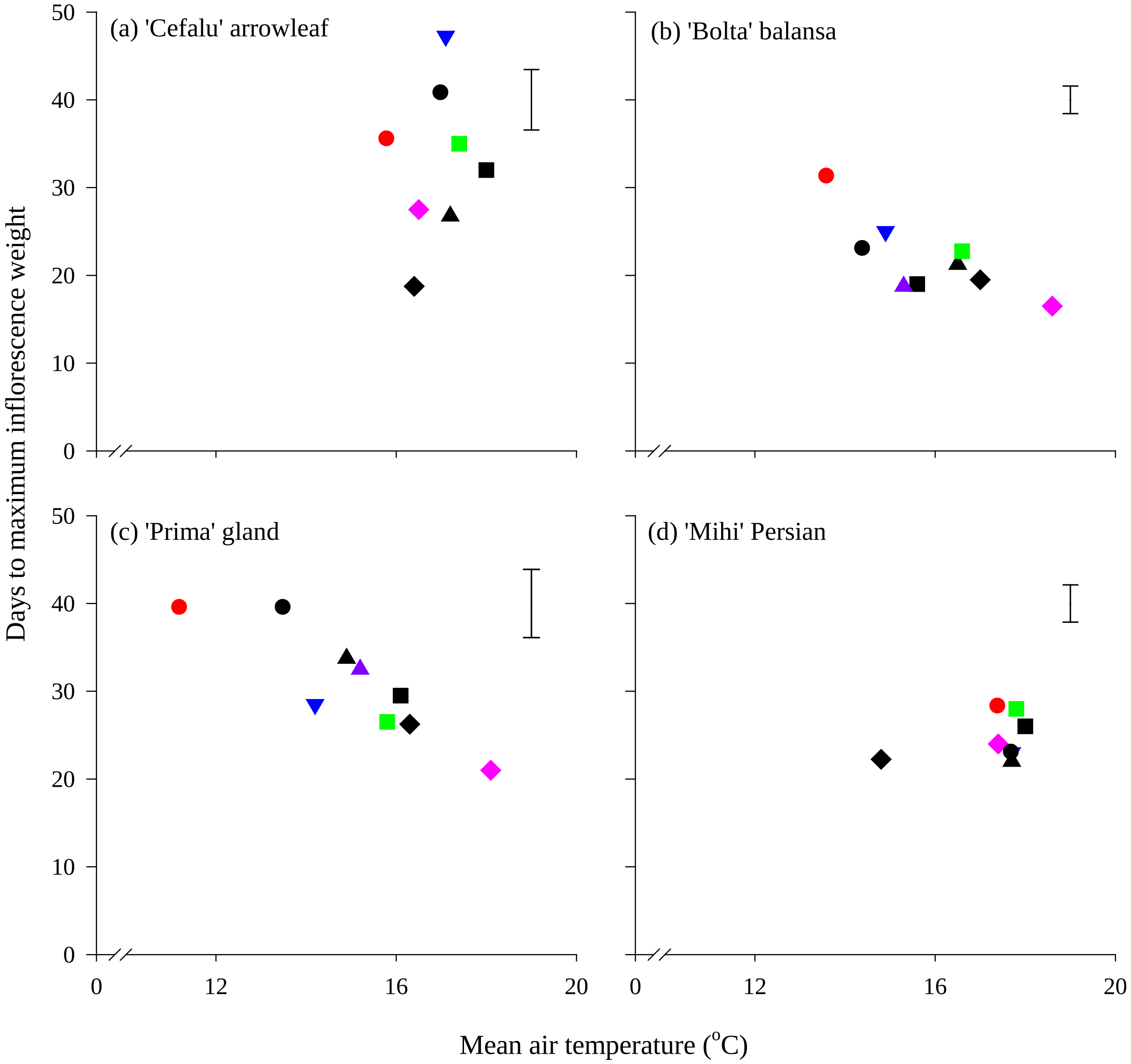


Figure 4.9 Days to maximum inflorescence weight for (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clover against mean temperature from pollination (stage 6) to maximum inflorescence weight sown on 10 different dates at Lincoln University, Canterbury, New Zealand. SD1 (●), SD2 (●), SD3 (▼), SD4 (▲), SD5 (■), SD6 (■), SD7 (◆), SD8 (◆), SD9 (▲). Error bars represent the maximum standard error of the mean. For actual sowing dates (SD), see Table 3.3.

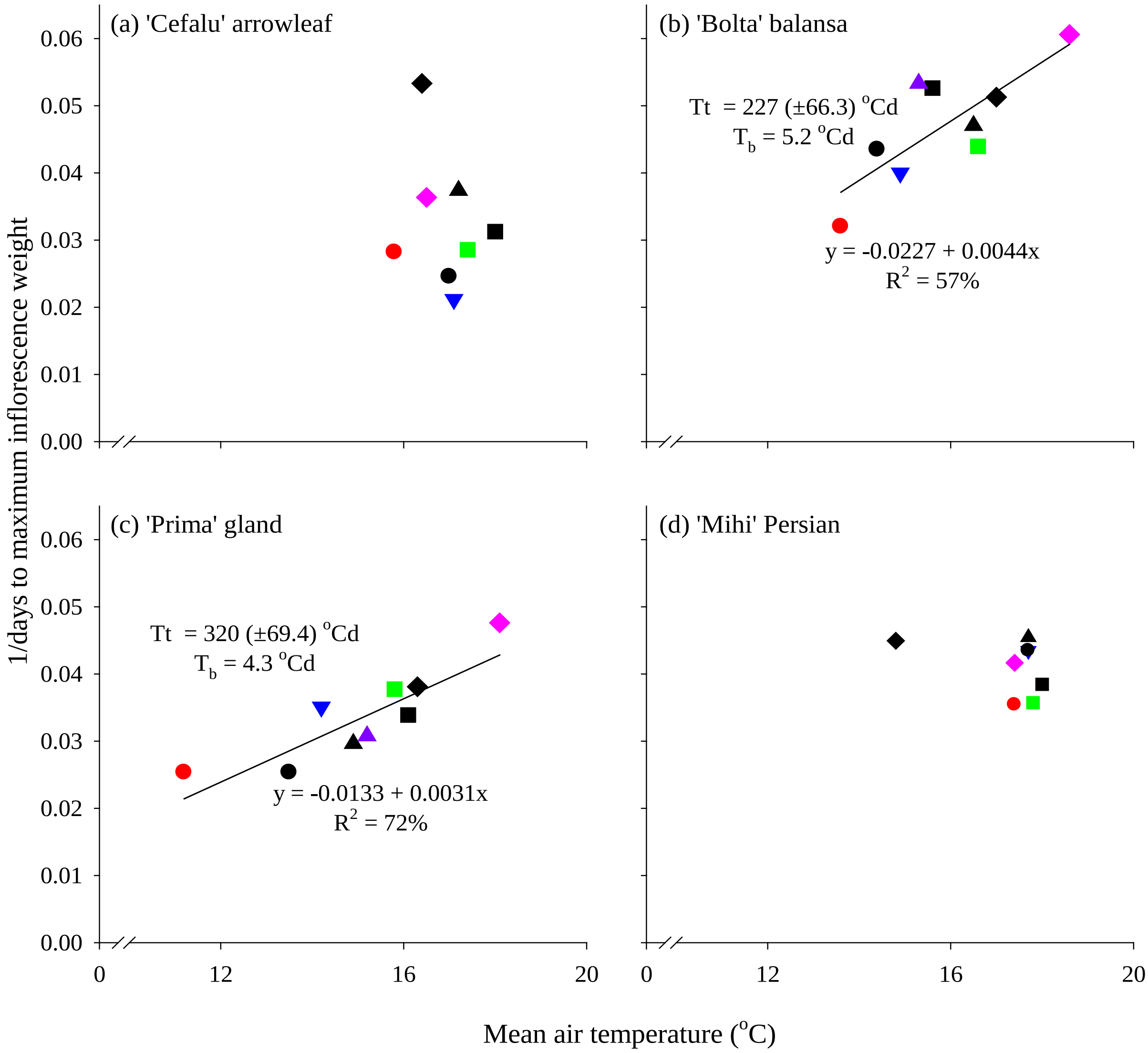


Figure 4.10 Rate of seed filling for (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clover against mean temperature from pollination (stage 6) to maximum inflorescence weight sown on 10 different dates at Lincoln University, Canterbury, New Zealand. SD1 (●), SD2 (●), SD3 (▼), SD4 (▲), SD5 (🟩), SD6 (■), SD7 (◆), SD8 (◆), SD9 (▲). For actual sowing dates (SD), see Table 3.3.

4.5.5 Revised reproductive development scale

In each species, the visual reproductive development scale was revised after all stages had been quantified. The revised scale (Table 4.10 to Table 4.13) highlighted chronologically the main events that occurred in the seed development process. The first two stages indicate seedling emergence (Stage 1) and the beginning of the flowering phase (Stage 2). Stage 3 indicates the inflorescence had reached full flower and was ready for pollination. Stage 4 showed the formation of pods within the inflorescence. Stage 5 indicated physiological maturity where seed filling actually stopped. Stage 6 marked harvest maturity where all seeds have dried and are ready for harvest. For ‘Prima’ gland and ‘Mihi’ Persian clover inflorescences, Stage 5 also coincided with their harvest maturity.

Table 4.10 Field applicable visual scale outlining the development of individual ‘Cefalu’ arrowleaf clover inflorescences

1	The plant has emerged
2	The inflorescence bud is visible in the axil of a leaf
3	Full flower – 100% of corolla have the standard unfolded from the wings
4	50% of pods are formed in the inflorescence
5	50% of seeds turned red/brown
6	100% of seeds turned red/brown (5 YR 5/10, 2.5 YR 3/8, 10 R 3/2)

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

Table 4.11 Field applicable visual scale outlining the development of individual ‘Bolta’ balansa clover inflorescences

1	The plant has emerged
2	The inflorescence bud is visible in the axil of a leaf
3	Full flower – 100% of corolla have the standard unfolded from the wings
4	Pods are visible within inflorescence
5	100% of pods are yellow
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.

Table 4.12 Field applicable visual scale outlining the development of individual ‘Prima’ gland clover inflorescences

1 The plant has emerged
2 The inflorescence bud is visible in the axil of a leaf
3 Full flower – 100% of corolla have the standard unfolded from the wings
4 Pods are visible within inflorescence
5 Seeds are hard and 100% of seeds are yellow (2.5 Y 8/6 to 5 Y 8/8)

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

Table 4.13 Field applicable visual scale outlining the development of individual ‘Mihi’ Persian clover inflorescences

1 The plant has emerged
2 The inflorescence bud is visible in the axil of a leaf
3 Full flower – 100% of corolla have the standard unfolded from the wings
4 Pods are visible within inflorescence
5 Pods turned brown. First sign of seeds change colour

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

4.6 Conclusion

The experiment described in this chapter has quantified the time of flowering for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers. Specific conclusions were:

1. Plants that emerged into an increasing photoperiod had a lower thermal time requirement for flowering compared with those that emerged into a decreasing photoperiod.

2. Time to flowering was accurately quantified by thermal time when the duration and direction of photoperiod at seedling emergence was accounted for. For ‘Bolta’ balansa and ‘Prima’ gland clovers, thermal time calculation for phenology from pollination to crop maturity used a T_b of 5.2 and 4.3 ^oC, respectively.

3. The minimum absolute photoperiod when flowering commenced was 11.3 hours for ‘Cefalu’ arrowleaf, 12.6 hours for ‘Bolta’ balansa, 10.3 hours for ‘Prima’ gland and 15.4 hours for ‘Mihi’ Persian clover.

4. The duration from pollination to physiological maturity was 274-689 ^oC d for ‘Cefalu’ arrowleaf, 185 ^oC d for ‘Bolta’ balansa, 256 ^oC d for ‘Prima’ gland and 425 ^oC d for ‘Mihi’ Persian clover.

5. Physiological maturity occurred when 50% of seeds had turned red/brown for ‘Cefalu’ arrowleaf, 100% of pods turned yellow for ‘Bolta’ balansa, 100% of seeds are yellow and hard for ‘Prima’ gland and pods turned brown with the first sign of colour changed in seeds for ‘Mihi’ Persian clover.

6. Photoperiod sensitivity commenced at the time of the first trifoliate leaf appearance.

5 Validation of the field experiments

5.1 Introduction

In Chapters 3 and 4, thermal time requirements for vegetative and reproductive development were quantified from a field experiment with 10 sowing dates across 2010-2011. Equations that describe the relationships between phenology and temperature and photoperiod were derived from this single location. In this chapter, additional experiments from 2011-2012 at different locations were used to validate the derived models for thermal time requirements for plant development stages. For some phases, notably emergence and first leaf appearance, the field experiment created a limited set of temperature values so controlled environments were used to extend the range. A controlled environment chamber (CEC) and pots in the nursery at Lincoln University were used for validation plus first year regeneration plots from the previous field experiment at Lincoln University and off-site field observations at (1) Castle Hill, Canterbury; (2) Lees Valley, Canterbury and (3) Seddon, Blenheim in the South Island of New Zealand.

Thus this chapter aims to validate the phenology models defined in Chapters 3 and 4 using these experiments from different locations and conditions. This evaluation will determine the accuracy of these models in prediction of phenology of annual clovers over a wider range of environments.

5.2 Materials and methods

5.2.1 Controlled Environment (five temperature regimes)

5.2.1.1 Experimental design

In each experiment, a completely randomised design was initiated with the four annual clover species (‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian) in four replicates. Each experiment was conducted in a CEC at one of five different temperature regimes: 9/6, 15/6, 20/10, 25/15, and 30/20 ^{o}C.

5.2.1.2 Husbandry

Plastic 4.5 L containers were filled with bark and pumice (4:1 by volume) potting mix containing 1 g/L Osmocote Plus (15% N, 5% P, 11% K), trace elements and 1 g/L dolomite lime (11% Mg, 24% Ca). In each pot, 50 seeds of the same four species used in calibration experiments (Chapter 3) were placed on the potting mix and then covered with a layer of 10 mm of potting mix. All pots were placed in a plant growth chamber (Conviron PGV36, Winnipeg, Canada) at the programmed temperatures. An 8/8 h temperature and photoperiod regime was used with 4 h transitions between day and night. The chamber was lit with a combination of 45 incandescent (Sylvania, 40 W) and 30 flourescent (Sylvania, 6 x 115 W and 24 x 215 W) lamps. Light reaching the plant canopies had a photosynthetic photon flux density (PPFD) of 448 ± 9 μ moles/m^2/s. In a natural environment, this is equivalent to overcast weather or a winters day. Full sunlight (PAR) is about 2200 μ moles/m^2/s in mid summer (Hay and Walker, 1989). Relative humidity ranged from 50 to 70%. Pots were watered daily at ~ 500 mL per pot and re-randomised every 14 days. Plants were thinned as required to reduce competition for moisture, nutrients and light. In each pot, 10 plants were marked for detailed measurement.

5.2.1.3 Measurements

5.2.1.3.1 Soil and air temperature

Four temperature sensors (Thermistors KTY-110) were used to measure temperatures within the growth chamber. Three sensors were placed within the soil at 10 mm depth and one at ~

0.4 m above ground level, protected by an aluminium tube. Temperatures were logged every 15 minutes with a HOBO data logger (Onset Computer Corporation) to determine daily mean temperatures. Due to variations between programmed temperature settings and the actual temperatures recorded by the temperature sensors (Figure 5.1), the latter were used for all analyses (Table 5.1).

Table 5.1 Comparison between programmed temperature settings and mean daily temperature as measured by temperature sensors in the Conviron PGV36 controlled environment chamber at Lincoln University, New Zealand in 2012.

Temperature setting (^{o}C)	Targeted mean daily temperature (^{o}C)	Mean daily temperature (^{o}C) as measured by temperature sensors	
		Air	Soil (10 mm)
9/6	7.5	7.9	10.6
15/6	10.5	11.0	13.2
20/10	15	15.6	17.0
25/15	20	20.4	20.7
30/20	25	25.4	23.9

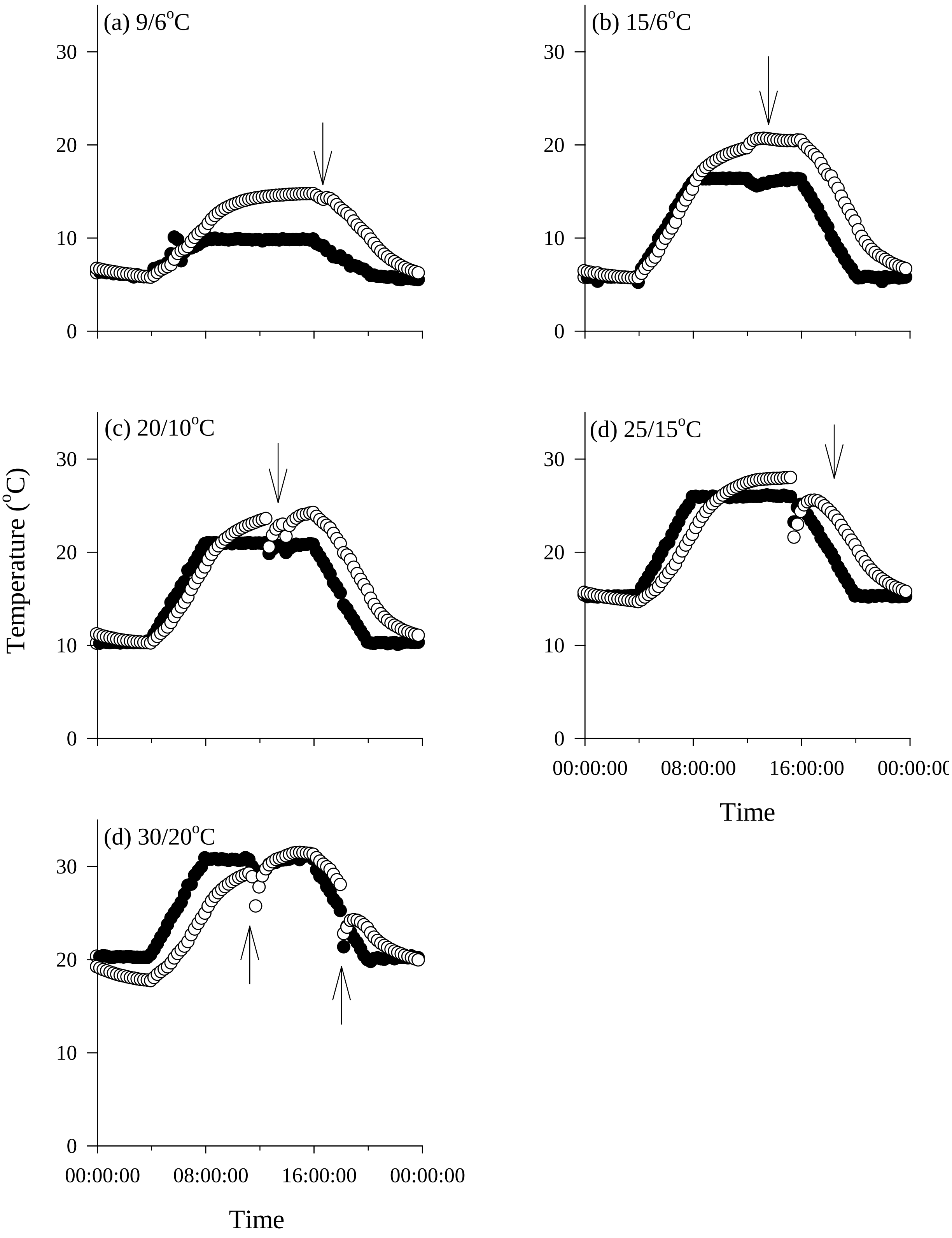


Figure 5.1 The air (●) and soil (○) temperatures change recorded by the temperature sensors over a 24 hour period in a controlled environment chamber at Lincoln University, New Zealand in 2012. Arrows indicate watering of crops in all chambers.

5.2.1.3.2 Emergence and first leaf appearance

Emerged seedlings were recorded daily until seedlings ceased to emerge. Time to 50% emergence and first leaf appearance were determined as described in Section 3.2.2.5.3.

5.2.1.3.3 Leaf appearance

The number of emerged leaves was counted at 3-7 day intervals on 10 marked plants per pot until 'Cefalu' arrowleaf clover (i.e. the slowest species) plants had produced their first axillary leaf. The leaf appearance rate (leaves/day) was determined from least squares regression analysis of the number of leaves on the main stem against days after first leaf appearance. The phyllochron (^{o}C d/leaf) was calculated from the regression of leaf appearance rate against mean air temperatures of each experiment. Because photoperiod setting was constant at 16 h in all experiments, only a single phyllochron was determined. The time to axillary leaf appearance was determined following procedures described in Section 3.2.2.5.4.

5.2.2 Experiments in glasshouse nursery, Lincoln University

5.2.2.1 Sowing dates

The aim of the glasshouse experiments was to produce plants at times not covered in the field experiment. Specifically there were gaps in the phyllochron and time to flowering data between the sowing dates of 19 January (photoperiod at 1st trifoliate leaf: 15.4 hours) and 26 February (photoperiod at 1st trifoliate leaf: 13.0 hours) for 'Cefalu' arrowleaf, 'Bolta' balansa and 'Prima' gland clovers. These were part of the validation experiments. In contrast, the sowing of 'Mihi' Persian clover was a repeat of the field experiments for sowing dates 9 (20 December 2010) and 10 (19 January 2011) in Iversen 9 field because these plants died in winter 2011 before 50% of the marked plants had a chance to flower. They were therefore considered as calibration experiments. Based on the results for time to flowering (Section 4.4.3), each species was sown with four replicates on different dates (Table 5.2).

Table 5.2 Sowing dates to quantify the phyllochron and time to flowering of four annual clovers sown at the glasshouse nursery, Lincoln University, New Zealand.

Species	Sowing date
‘Cefalu’ arrowleaf	7 February 2012
‘Bolta’ balansa	24 January 2012
‘Prima’ gland	7 February 2012
‘Mihi’ Persian	20 December 2011 and 19 January 2012

All pot experiments were established on the ground in an open air glasshouse nursery at Lincoln University. A pot experiment in a glasshouse was used rather than the field because during the summer sowings, moisture can limit seedling emergence, as seen in Figure 3.9. Thus, the glasshouse offered greater control of watering opportunities and ensured exposure to the required photoperiod and air conditions.

5.2.2.2 Husbandry

A plastic container measuring 460 x 300 x 100 mm was filled with bark and pumice potting mix (Section 5.2.1.2). Seeds of annual clovers were sown at 0.25 g/pot (~11 kg/ha) for ‘Cefalu’ arrowleaf, 0.13 g/pot (~7 kg/ha) for ‘Bolta’ balansa, 0.16 g/pot (~7 kg/ha) for ‘Prima’ gland and 0.14 g/pot (~8.5 kg/ha) for ‘Mihi’ Persian clover. Immediately after sowing, the pots were gently watered by hand. Pots were then watered twice daily in the morning (by automatic sprinkler system) and in afternoon (manually), to ensure the surface remained moist and soil ‘capping’ did not occur.

5.2.2.3 Measurement

5.2.2.3.1 Air temperature and photoperiod

The temperature sensor (Thermistors KTY-110) was placed at 400 mm above the ground, was protected by an aluminium shield and logged every hour with a HOBO data logger (Onset Computer Corporation) to define the daily maximum and minimum temperatures (Section 3.2.2.5.1). Photoperiod calculation was described in Section 3.2.2.5.2.

5.2.2.3.2 Emergence and first leaf appearance

Date of seedling emergence and first leaf appearance were determined by visual assessment when 50% of the seeds sown had visible cotyledons and when 50% of the seedlings population had their first leaf appeared.

5.2.2.3.3 Leaf appearance

The leaf appearance was measured only on ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers. The number of leaves on the main stem was counted at weekly intervals until they had produced 10 leaves. The phyllochron and time to first axillary leaf appearance were also calculated (Section 3.2.2.5.4).

5.2.2.3.4 Time to flowering

The time to flowering was determined when 50% of the plant population within a pot had their first bud visible.

5.2.3 Experiments in Iversen 9 field, Lincoln University in year 2011

The experiments described in this section consist of (1) additional sowing for ‘Mihi’ Persian clover and (2) first year regeneration of annual clovers from crops sown in 2010.

5.2.3.1 Additional sowing for ‘Mihi’ Persian clover

The aim of this sowing was to complete the annual data for time to flowering between the sowing dates of 25 September (photoperiod at 1st trifoliate leaf: 14.6 hours) and 9 November (photoperiod at 1st trifoliate leaf: 16.2 hours) and therefore was part of the calibration data set. Based on the result of time to flowering (Section 4.4.3), sowing was on 17 October 2011 at a target photoperiod at 1st trifoliate leaf of 15.5 hours.

5.2.3.1.1 Design and sowing

The experiment was a randomized complete block design with four replicates. Seeds of ‘Mihi’ Persian clover of the same line as all previous experiments were hand sown at 5 kg/ha.

Each plot size measured 3 x 2 m. Land preparation and management of seedlings were performed as described in Section 3.2.2.4.

5.2.3.1.2 Measurements

Measurements of phenology and calculations of thermal time and photoperiod were performed in the same manner as described previously.

5.2.3.2 First year regeneration of annual clovers sown in 2010

The aim of this experiment was to quantify the duration from when shattered seeds dropped until their subsequent emergence and time to flowering.

After crops completed their life cycle, the dead herbage was removed using a rotary mower on 29 April 2011 to facilitate seedling recruitment. The date of the earliest seedling emergence within a subplot and their date of first bud visible were recorded. Air temperature was measured by the temperature sensor installed in the experimental site (Section 3.2.2.5.1). Photoperiod was determined as in Section 3.2.2.5.2.

5.2.4 Experiments off-site in the South Island of New Zealand

The aim of these experiments was to quantify time to flowering and phyllochron of annual clovers when grown in different dryland locations of higher altitudes and different latitudes than those used for the calibration exercise. Three commercial farm locations in the South Island were used (Table 5.3): (1) Castle Hill, Canterbury (2) Lees Valley, Canterbury (3) Breach Oak, Blenheim. These were remote locations where Castle Hill was ~96 km (1.5 hours drive), Lees Valley was ~102 km (2 hours drive) and Breach Oak was ~303 km (4 hours drive) from Lincoln University. As a consequence, these sites were visited less frequently than on-station experiments.

5.2.4.1 Location description

These locations provided a similar photoperiod but different temperature range due to their altitude. Both Castle Hill and Lees Valley are located in the Canterbury high country of the

South Island. Specifically, the experimental area in the Lees Vallley was located in Block 2 of an established 10 hectare experimental site. For Breach Oak, the experimental area was located in a dryland farm in Seddon, Blenheim. The description for each location is summarized in Table 5.3.

Table 5.3 Geographical coordinate, altitude, soil type and vegetation in the Castle Hill, Lees Valley and Breach Oak.

Location	Geographical coordinate, altitude	Soil type	Vegetation
Castle Hill, Canterbury	43^o 14’S, 171^o 43’E, 700 m a.s.l.	Craigieburn silt loam (NZ classification: Upland and high country yellow-brown earths) (D.S.I.R., 1968)	*Hieracium pilosella* and browntop (*Agrostis tenuis*)
Lees Valley, Canterbury	43^o 8’S, 172^o 11’E, 434 m a.s.l.	Ashwick shallow silt loam (NZ classification: Yellow-grey to yellow-brown earths intergrade), with stony profile (D.S.I.R., 1968)	White clover, red clover, Caucasian clover, Subterranean clover, lucerne, chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*)
Breach Oak, Blenheim	41^o 43’S, 173^o 58’E, 198 m a.s.l.	Flaxbourne hill soil, silt loam (NZ classification: Yellow-grey earths) (D.S.I.R., 1968)	Plantain, lucerne, white clover, subterranean clover and other pasture species

5.2.4.2 Soil test

Soil tests were conducted on 2nd December 2011 for Castle Hill, 11th August 2010 for Lees Valley and 15th October 2010 for Breach Oak soils (Table 5.4).

Table 5.4 Soil test (0-75 mm) results for Castle Hill, Lees Valley and Breach Oak experimental locations.

Analysis	Results			Recommended range
	Castle Hill	Lees Valley	Breach Oak	
pH	5.8	5.6	5.6	5.8 - 6.2
[1]Olsen Phosphorus	20	18	9	20 - 30
[2]Calcium	5.8	7.6	8.1	3.0 -9.0
[2]Magnesium	0.83	0.7	4.0	1.0 – 1.5
[2]Potassium	0.57	0.80	1.19	0.30 – 0.50
[2]Sodium	0.06	0.05	0.26	0.20 -0.40
[2]C.E.C	19	20	23	12 - 25
[3]Sulphate Sulphur	8	-	5	10 - 12

Note: In the Castle Hill, 2 t/ha of lime and 1 t/ha of superphosphate was applied on 1st February 2011. The S.I. unit for [1,2,3] are µg/mL, me/100g and mg/kg respectively.

5.2.4.3 Meteorological conditions

5.2.4.3.1 Castle Hill, Canterbury

Monthly rainfall varied over the year and ranged from 23 mm in September to 120 mm in July (Figure 5.2). The mean daily air temperature ranged from 1 – 2 ^{o}C in July/August to 14 ^{o}C in January/February. The mean daily 10 mm soil temperature ranged from 0.6 – 2 ^{o}C in July/August to 16.5 ^{o}C in January/February.

5.2.4.3.2 Lees Valley, Canterbury

Monthly rainfall averaged ~70 mm with the highest rainfall (140 mm) in October (Figure 5.3). The driest month was September with 37 mm of rainfall. Daily mean air temperature ranged from 16 ^{o}C in January/February to 3 ^{o}C in July/August. Daily mean soil temperature was highest in January/February (~19 ^{o}C) and lowest in July/August (3 ^{o}C).

5.2.4.3.3 Breach Oak, Blenheim

Monthly rainfall was variable over the year (Figure 5.4) and ranged from 97 mm in May to 0.6 mm in January. In the majority of months, rainfall was below 50 mm. February was the warmest month of the year with daily mean air temperature of 18.4 °C. July was the coldest month with daily mean air temperature of 4.8 °C. Daily mean soil temperature ranged from 20.5 °C in February to 7 °C in July.

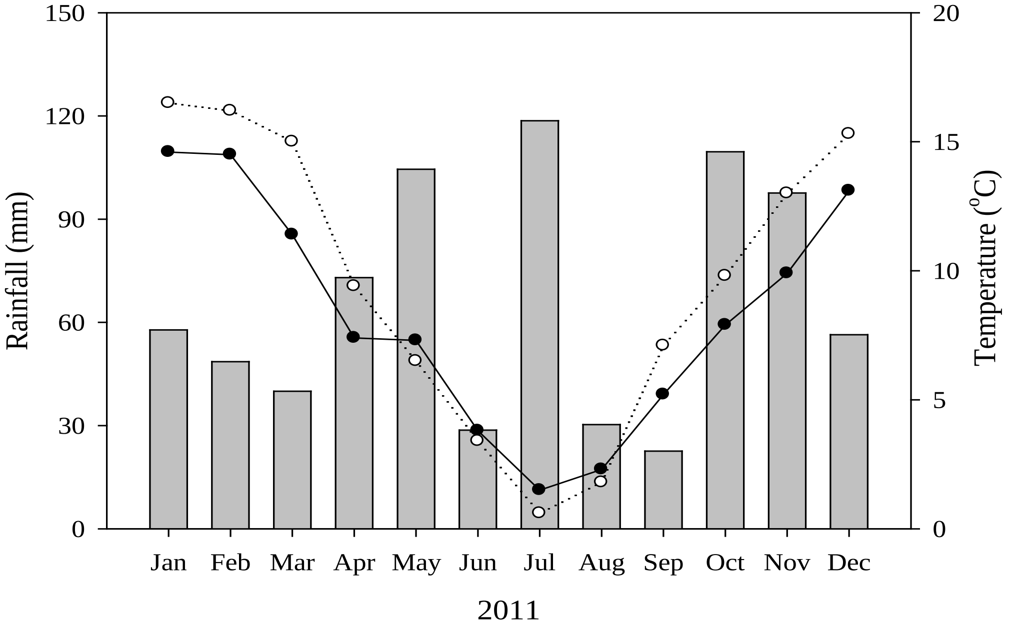


Figure 5.2 Monthly rainfall (bars) and mean daily air (●) and 10 mm soil (○) temperatures for 2011 at Castle Hill, Canterbury, New Zealand. Rainfall data were obtained from Castle Hill Village meteorological station, Canterbury, New Zealand.

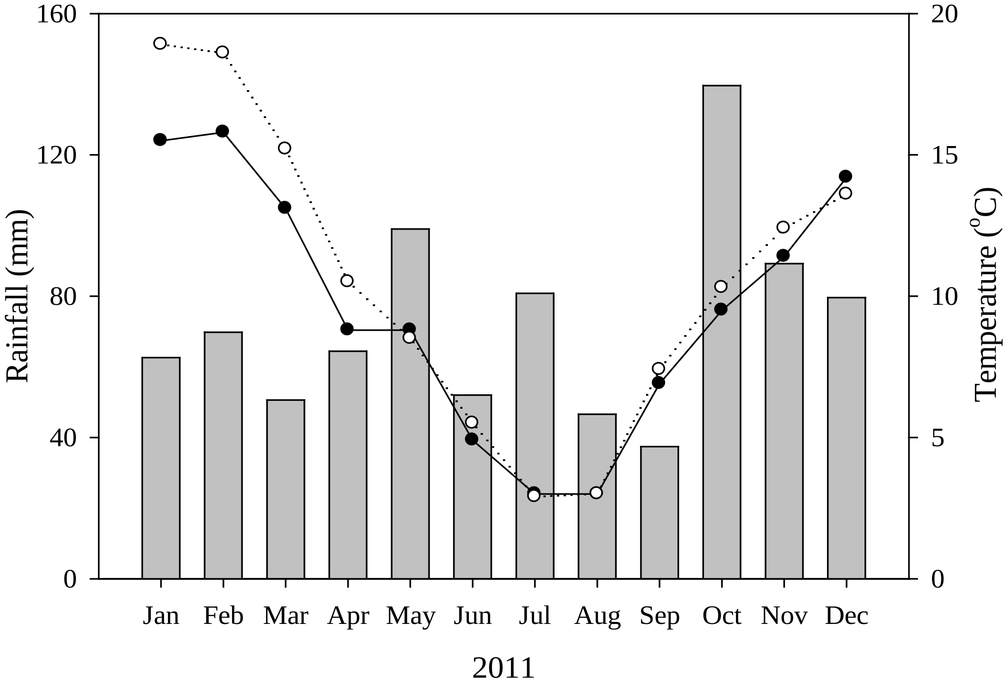


Figure 5.3 Monthly rainfall (bars) and mean daily air (●) and 100 mm soil (○) temperatures for 2011 at Lees Valley, Canterbury, New Zealand.

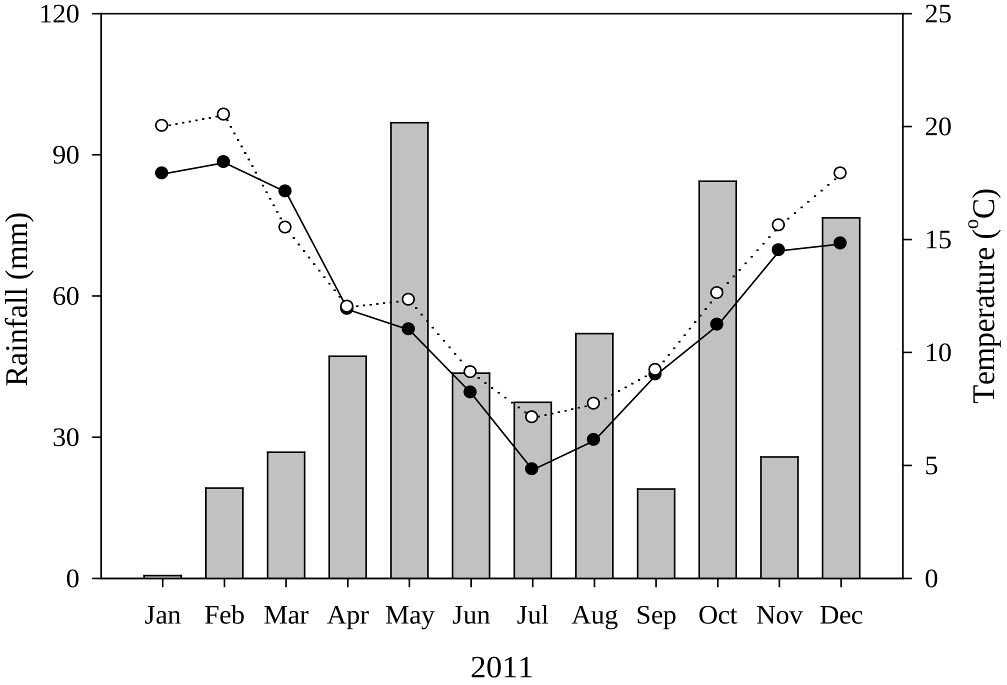


Figure 5.4 Monthly rainfall (bars) and mean daily air (●) and 10 mm soil (○) temperatures for 2011 at Breach Oak, Seddon, Blenheim, New Zealand.

5.2.4.4 Experimental design and sowing

5.2.4.4.1 Castle Hill, Canterbury

The design was a randomized complete block with two replicates and four clover species (‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian) sown on 11 March 2011. Each plot measured 1 x 3 m, giving an area of 3 m^2. Bare seeds were broadcast by hand as pure stands at 20 kg/ha for each clover species. Seeds were sown with Group C inoculants. Plots were sprayed with Buster (a.i. 200 g/L glufosinate ammonium) on 1st February 2011 and no irrigation was applied.

5.2.4.4.2 Lees Valley, Canterbury

A split-plot design experiment with four replicates was initiated on 29th March 2011. Main plots were four annual clover species (‘Cefalu arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian) while subplots were treatments (with and without inoculation). Each subplot measured 3 x 31 m, and these were separated by an area of 2 x 31 m of a Campeda/Leura subterranean clover mix (Figure 5.5).

Prior to sowing, the experimental area was lightly cultivated using harrows towed behind a quad bike. Bare seeds of four annual clover species were sown as pure stands at 20 kg/ha for each species. For each replicate, the uninoculated seeds were sown first, followed by the subterranean clovers mix and then inoculated seeds. The seeds were broadcast with a hand-held sower. The inoculant used in this experiment was moist peat Group C inoculant (sourced: Becker Underwood Pty. Ltd.). Each plot was sprayed with Buster at 11 L/ha on 16th March 2011.

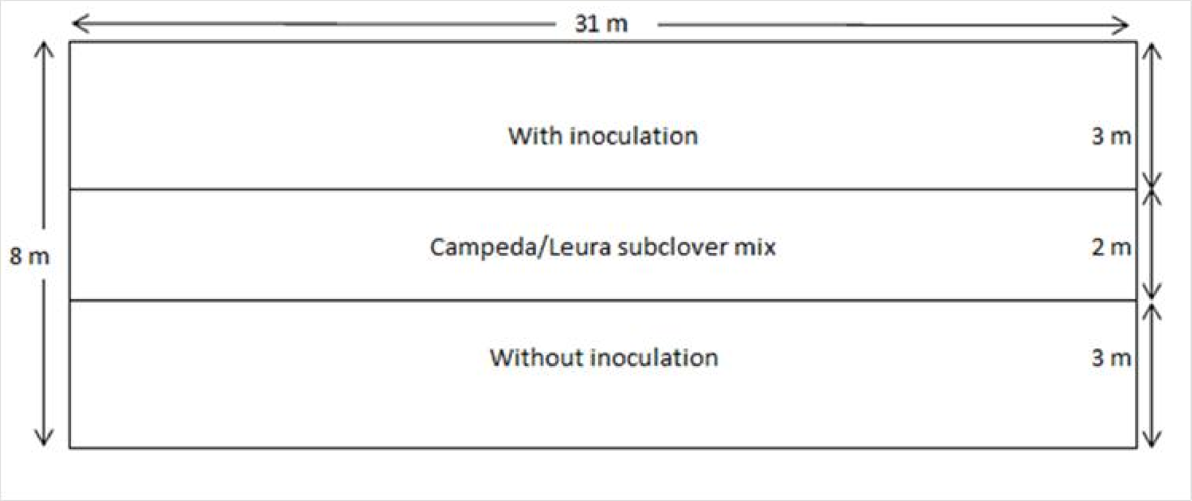


Figure 5.5 Subplots (with and without inoculation) layout within each replicate.

5.2.4.4.3 Breach Oak, Blenheim

The experiment was a randomized complete block design comprised of the four annual clover species (‘Cefalu arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian) and each replicate was located at the east and west side of the valley. The distance between each replicate was ~ 50 m. Each plot measured 1 x 1.5 m, giving an area of 1.5 m^2.

On 15th April 2011, bare seeds were broadcast as pure stands at 15 kg/ha for each species. Seeds were sown without inoculants. Plots were sprayed with Glyphosate 540 (a.i. 540 g/L glyphosate) at 5.5 L/ha and Hammer (240 g/L Carfentrazone – ethyl) at 100 mL/ha on 3rd March 2011.

5.2.4.5 Measurements

5.2.4.5.1 Temperature and photoperiod

Temperature sensors were placed at each location (Table 5.5). Thermal time and photoperiod calculations were performed as described in Section 3.2.2.5.1 and 3.2.2.5.2.

5.2.4.5.2 Emergence and first leaf appearance

Because of the remoteness of each location, emergence was not observed. Therefore, the date of first leaf appearance was calculated from extrapolation of the number of leaves against time (in days). Emergence was then estimated based on the time of rainfall after sowing. Because no irrigation was applied following sowing and based on the rainfall data, emergence was predicted to have occured within 5-7 days after rainfall.

5.2.4.5.3 Leaf appearance

Leaf appearance was measured on different dates at each location (Table 5.5). On each date, 10 plants per plot were destructively harvested; the number of leaves on the main stem and total number of leaves were recorded. The phyllochron was calculated according to Section 3.2.2.5.4.

5.2.4.5.4 Time to axillary leaf appearance

The appearance of axillary leaves and date of initial axillary leaf production were estimated based on the number of nodes to axillary leaf derived from the Iversen 9 experiment result (Table 3.10). For autumn sown plants (depending on dates), the first axillary leaf was initiated after the appearance of five nodes for ‘Cefalu’ arrowleaf, 4-6 nodes for ‘Bolta’ balansa, 4 nodes for ‘Prima’ gland and 4-5 nodes for ‘Mihi’ Persian clover (Table 3.10). The date of first axillary leaf appearance was calculated from interpolation of the number of leaves against time.

5.2.4.5.5 Time to flowering

Time to flowering was inspected at three week intervals starting from September 2011. Time to flowering was defined as the time when any plant within a plot had their first bud visible.

Table 5.5 Dates of measurement of temperature and leaf appearance at Castle Hill, Lees Valley and Breach Oak experimental locations.

Location	Measurement dates	
	Temperature	Leaf appearance
Castle Hill, Canterbury	Four temperature sensors: One sensor at 1 m above the ground, protected by an aluminium shield; two at 10 mm depth within the soil and one at 100 mm depth within the soil.	21st April, 1st June, 8th August, 21st October, 8th November and 2nd December 2011.
Lees Valley, Canterbury	Two temperature sensors: One sensor placed within the soil at 100 mm depth and another one at 1m above the ground, protected by a solar radiation shield (RS3).	24th May, 3rd August, 7th September, 27th September, 9th October, 31st October and 29th November 2011.
Breach Oak, Blenheim	Temperature sensors were placed on both east and west sides of the valley. On each side, one sensor was placed at 1.2 m above the ground and three were placed in the soil, with two at 10 mm depth and one at 100 mm depth.	20th May, 30th August, 14th October and 13th December 2011.

5.2.5 Reanalysis from published data

The data published from Monks (2009) on ‘Bolta’ balansa clover sown at Lincoln University in the year 2005-2006 were also re-analyzed with some modifications: base temperature (T_b) set to 0 ^oC, photoperiod calculation to include civil twilight and response to photoperiod at first trifoliate leaf stage. These data were also used to test the accuracy of the flowering model developed from the Iversen 9 experiment in 2010-2011.

5.2.6 Validation of the model

Model validation compared the estimated and observed values for each phenology study from independent data sets (from experiments in locations other than Iversen 9 field, Lincoln University). The estimated values were obtained by using the calibrated linear model from Iversen 9 field experiment. The differences between observed and estimated value was tested by the root mean square deviation (RMSD):

Equation 5.1

$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{n}(x_{obs} - x_{pre})^2}{n}}$$

where x_{obs} and x_{pre} is the observed and predicted value of development parameter (rate or thermal time) respectively, and n is the number of observations. An RSMD of <20% is regarded as an accurate prediction of the observed values.

5.3 Results

5.3.1 Seedling emergence

5.3.1.1 Controlled environment chamber (CEC)

Final emergence percentage ranged between 12 and 34% for ‘Cefalu’ arrowleaf, 32 and 65% for ‘Bolta’ balansa, 30 and 47% for ‘Prima’ gland and 79 and 95% for ‘Mihi’ Persian clover (Figure 5.6) across the five temperature regimes.

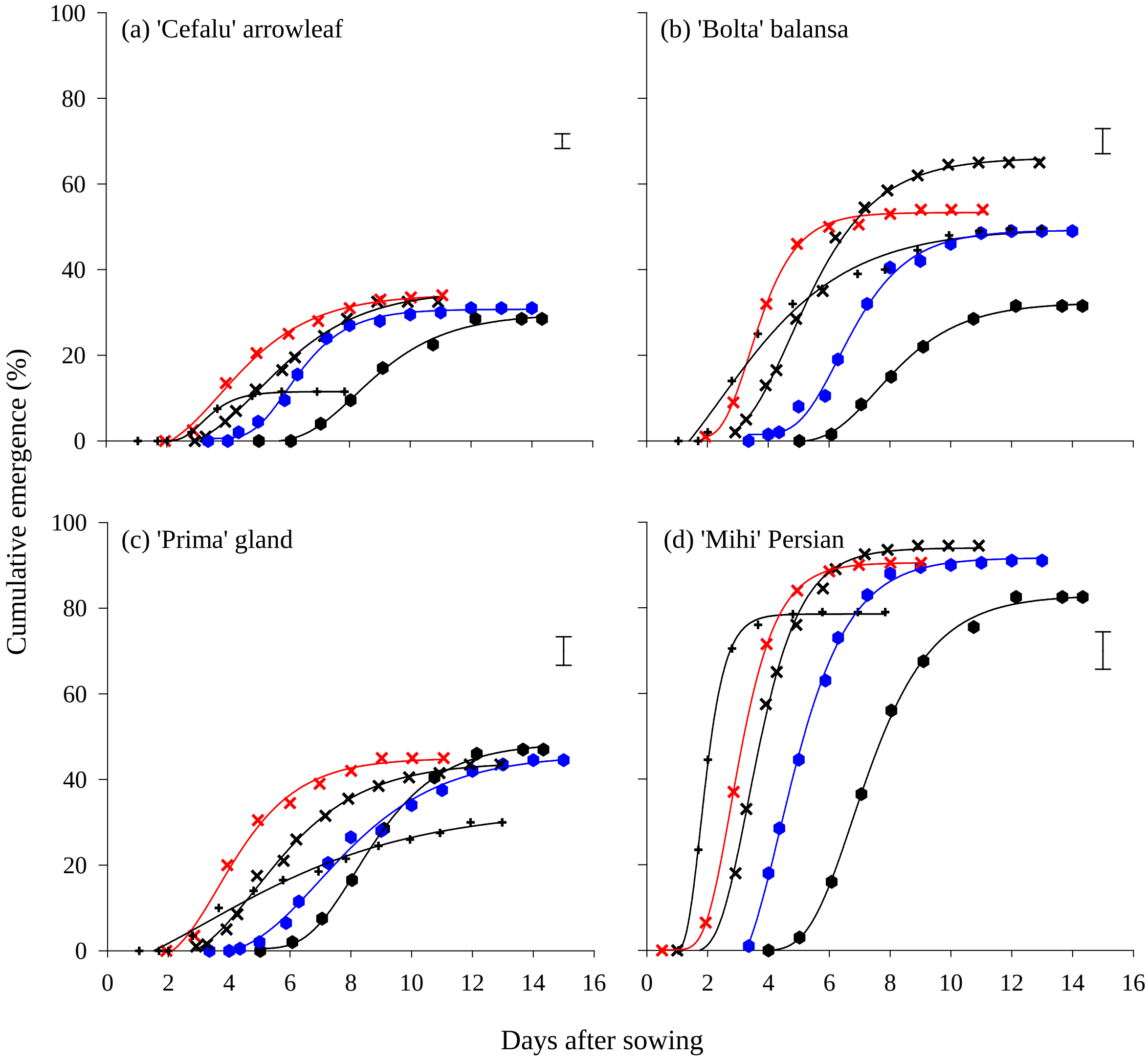


Figure 5.6 Cumulative emergence of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa , (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers at different mean soil (10 mm) temperatures in a controlled environment chamber at Lincoln University in 2012. (⬢) 10.6°C, (⬢) 13.2°C, (✕) 17.0°C, (✕) 20.7°C, (+) 23.9°C. Error bars represent the maximum standard error for the final number of emerged seedlings.

The number of days to 50% of final emergence decreased as mean soil (10 mm) temperature increased up to 23.9°C for both 'Cefalu' arrowleaf and 'Mihi' Persian clovers and up to 20.7°C for both 'Bolta' balansa and 'Prima' gland clovers. Linear regression between the emergence rate and mean soil temperature enabled T_b and Tt to be calculated (Table 5.6). Above a T_b of 0°C, Tt requirements for emergence were 93 °C d for 'Cefalu' arrowleaf, 83 °C d for 'Bolta' balansa, 95 °C d for 'Prima' gland and 56 °C d for 'Mihi' Persian clovers.

Table 5.6 Base temperature (T_b) and thermal time (Tt) requirements for 50% emergence of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown in a controlled environment chamber at Lincoln University in 2012.

Species	T_b (°C)	Tt (°C d)	R^2 (%)	[1]Tt (T_b=0) (°C d)
'Cefalu' arrowleaf	-2.3	106	83	93
'Bolta' balansa	2.7	70	94	83
'Prima' gland	1.2	88	82	95
'Mihi' Persian	6.1	38	88	56
Maximum s.e.	0.47	12.4		2.9
95% c.i.	-9.8, 10.2			

[1] Analysis assumes a base temperature of 0°C. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

5.3.1.2 Validation of the field experiment

5.3.1.2.1 'Cefalu' arrowleaf

In the CEC experiment, 'Cefalu' arrowleaf clover sown at mean soil temperature regime from 10.6 – 23.9 °C required 93 °C d (T_b = 0 °C) to emerge (Table 5.6) compared with 88 °C d (T_b = 0 °C) in the calibration experiment with a mean soil temperature range between 5.3 and 19.1°C (Table 3.5). Thus, the calibration model provided a good fit (Figure 5.7a) with an RMSD value of 9.2%.

5.3.1.2.2 'Bolta' balansa

The emergence rate of 'Bolta' balansa clover in the CEC was described by a single relationship and had a thermal time requirement of 83 ^{o}C d (T_b = 0 ^{o}C) (Table 5.6). Emergence rate declined at the highest soil temperature of 23.9 ^{o}C which corresponded with the decrease in their germination rate at temperatures beyond 20 ^{o}C (Figure 3.6). In contrast, the estimated emergence rate described by the bi-linear relationship (Figure 5.7b) was 92 ^{o}C d (T_b = 0 ^{o}C) at mean soil temperature from 5.1 – 10.4 ^{o}C and 67 ^{o}C d (T_b = 0 ^{o}C) at mean soil temperature from 12.1 – 18.8 ^{o}C (Table 3.5). The emergence rate in the CEC at mean soil temperatures between 13.2 and 20.7 ^{o}C was lower than in the field calibration experiment (Figure 5.7b) with an RMSD of 25.5%.

5.3.1.2.3 'Prima' gland

The thermal time to emergence for 'Prima' gland clover sown in the CEC was 95 ^{o}C d; (T_b = 0 ^{o}C) and similar to the 96 ^{o}C d from the calibration experiment (Table 5.6 and Table 3.5). The calibration model gave an RMSD of 4.4% for the observed data in the CEC (Figure 5.7c).

5.3.1.2.4 'Mihi' Persian

The emergence rate for 'Mihi' Persian clover sown in CEC was described by a single relationship with thermal time requirement of 56 ^{o}C d (T_b = 0 ^{o}C) (Table 5.6). This was lower than the thermal time to emergence from the field experiment which were 93 ^{o}C d (T_b = 0 ^{o}C, from 5.2 – 12.1 ^{o}C) and 50 ^{o}C d (T_b = 0 ^{o}C, from 16.1 – 18.5 ^{o}C) (Table 3.5). The RMSD for emergence rate was 22.6% at 10.6 ^{o}C and 27.8% for mean soil temperature between 13.2 and 17.0 ^{o}C.

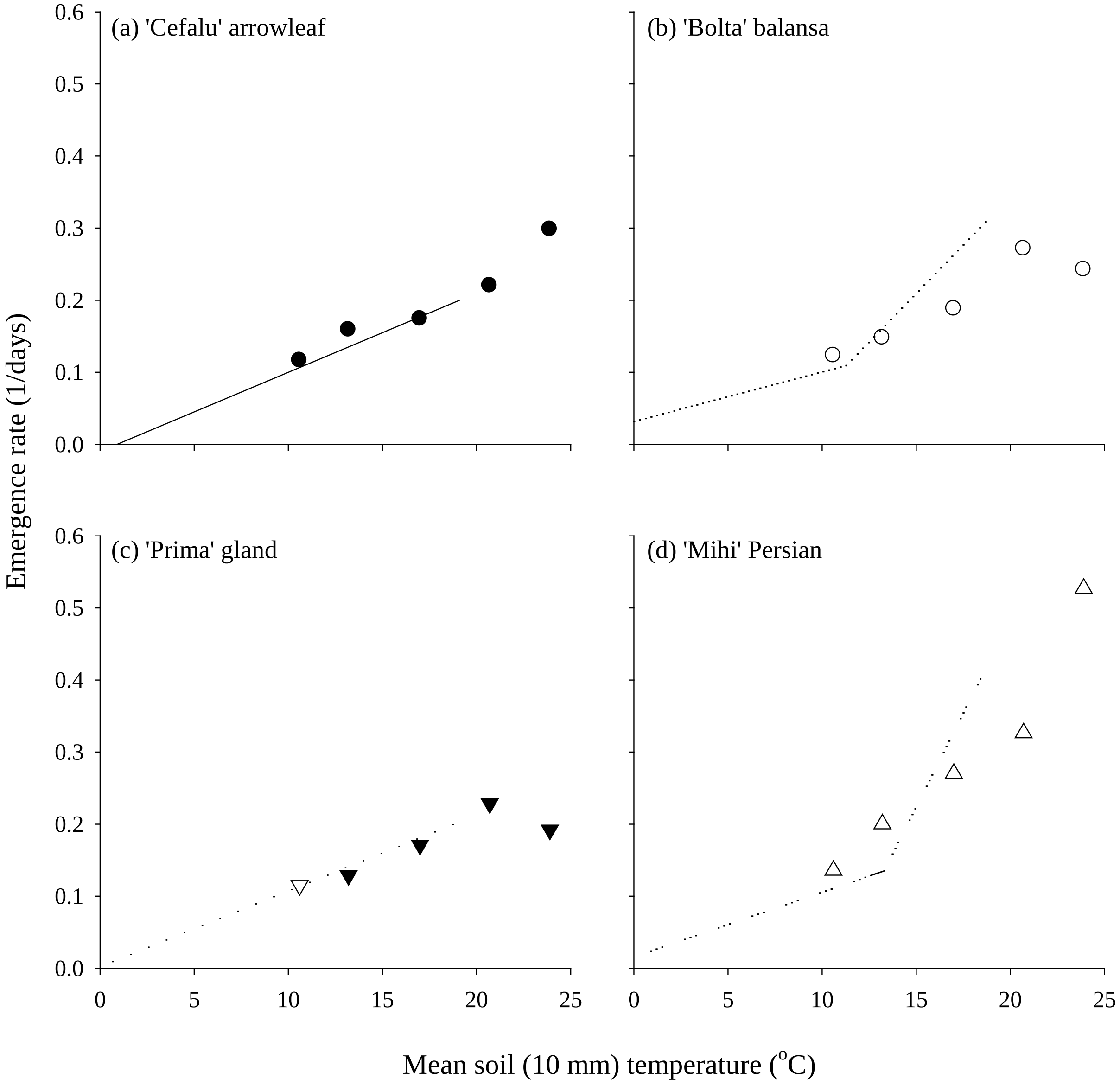


Figure 5.7 Calibrated (lines) and observed (symbols) emergence rate of (a) Cefalu' arrowleaf (●), (b) 'Bolta' balansa (○), (c) 'Prima' gland (▼) and (d) 'Mihi' Persian (△) clover sown in a controlled environment chamber at different mean soil (10 mm) temperatures at Lincoln University in 2012.

5.3.1.3 Fitted model using data from all locations

Following the validation of the emergence model from calibration experiment, the emergence data from both Iversen 9 field experiment and the CEC experiment were combined and the model again fitted to the larger data set (Figure 5.8). Above a T_b of 0 ^oC, thermal time to emergence was estimated at 90 ^oC d for 'Cefalu' arrowleaf and 96 ^oC d for 'Prima' gland clover in a single linear relationship (Table 5.7). For 'Bolta' balansa clover, the thermal time to emergence was 90 ^oC d (T_b = 0 ^oC) at mean soil temperature range between 5.1 and 10.6 ^oC. At higher soil temperature from 12.1 - 20.7 ^oC, 'Bolta' balansa clover seedlings took 75 ^oC d (T_b = 0 ^oC) to emerge. Similarly, 'Mihi' Persian clover took longer (87 ^oC d, T_b = 0 ^oC) to emerge at lower soil temperatures of 5.2 – 10.6 ^oC. As soil temperature increased from 12.1 – 23.9 ^oC, seedlings emerged earlier at 55 ^oC d (T_b = 0 ^oC).

Table 5.7 Base temperature (T_b) and thermal time (Tt) requirements for 50% emergence of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown at Iversen 9 field, Lincoln University in 2010-2011and controlled environment chamber at Lincoln University in 2012.

Species	Temperature range (^oC)	T_b (^oC)	Tt (^oCd)	R^2 (%)	[1]Tt (T_b=0 ^oC) (^oCd)
'Cefalu' arrowleaf	5.3 -20.7	0.2	89	83	90
'Bolta' balansa	5.1 -10.6	-2.4	115	76	90
	12.1 - 20.7	5.2	52	43	75
'Prima' gland	5.2 - 20.7	0.1	95	77	96
'Mihi' Persian	5.2 - 10.6	-0.2	88	75	87
	12.1 - 23.9	7.2	33	75	55
Max. s.e.		0.75	15.0		4.6
95% c.i.		-12.6,12.1			

[1]Analysis assumes a base temperature of 0^oC. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

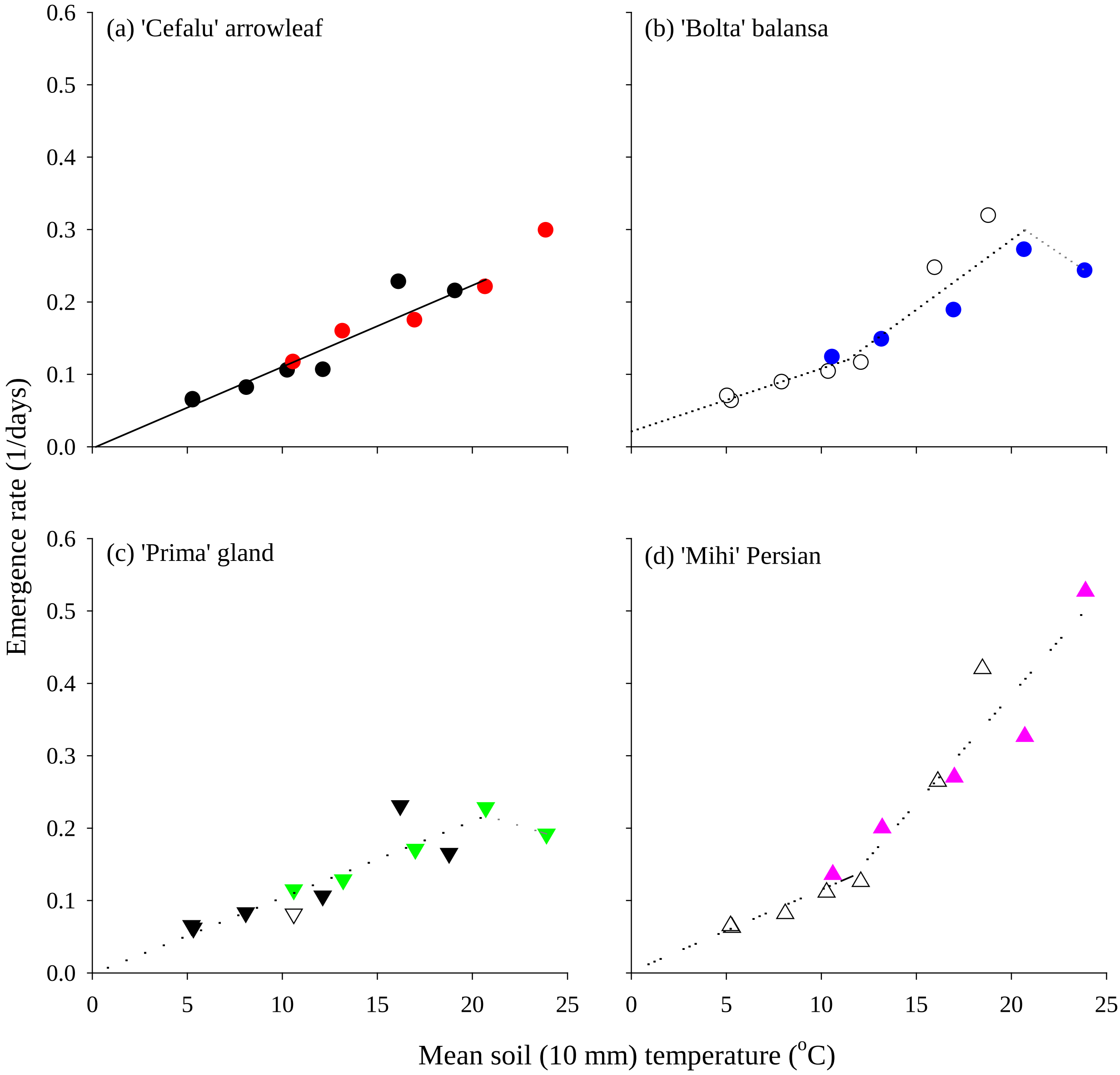


Figure 5.8 Emergence rate of (a) 'Cefalu' arrowleaf (●,●), (b) 'Bolta' balansa (○,●), (c) 'Prima' gland (▼,▼) and (d)'Mihi' Persian (△,▲) clovers at different mean soil (10 mm) temperatures at Iversen 9 field, Lincoln University in 2010-2011 (black and white symbols) and controlled environment chambers at Lincoln University in 2012 (coloured symbols).

5.3.2 First leaf appearance

5.3.2.1 Controlled environment chamber (CEC)

The number of days from sowing to first leaf (spade leaf) appearance decreased as mean soil (10 mm) temperature increased up to 23.9 ^{o}C for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Mihi’ Persian and up to 20.7 ^{o}C for ‘Prima’ gland clover. Linear regression between the rate of first leaf appearance and mean soil (10 mm) temperature enabled T_b and Tt to be calculated (Table 5.8). In all species, T_b was not different from 0 ^{o}C. Therefore, when T_b was set to 0 ^{o}C, the Tt requirement for first leaf appearance averaged 181 ^{o}C d for ‘Cefalu’ arrowleaf, 151 ^{o}C d for Bolta’ balansa, 168 ^{o}C d for ‘Prima’ gland and 135 ^{o}C d for ‘Mihi’ Persian clover.

Table 5.8 Base temperature (T_b) and thermal time (Tt) requirements for 50% first leaf appearance of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in controlled environments.

Species	T_b (^{o}C)	Tt (^{o}C d)	R^2 (%)	[1]Tt (T_b=0) (^{o}C d)
‘Cefalu’ arrowleaf	-1.2	195	97	181
‘Bolta’ balansa	3.6	118	98	151
‘Prima’ gland	2.2	145	92	168
‘Mihi’ Persian	4.4	103	96	135
Maximum s.e.	0.30	11.0		3.8
95% c.i.	-3.7, 6.7			

[1]Analysis assumes a base temperature of 0^{o}C. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

5.3.2.2 Validation of the field experiment

In all species, the rate of first leaf appearance in the CEC experiment was faster than from the calibration experiment (Figure 5.9). The RMSD calculated as a percentage of estimated mean was 20.1% for ‘Cefalu’ arrowleaf, 16.9% for ‘Bolta’ balansa, 5.5% for ‘Prima’ gland and 28.4% for ‘Mihi’ Persian clover.

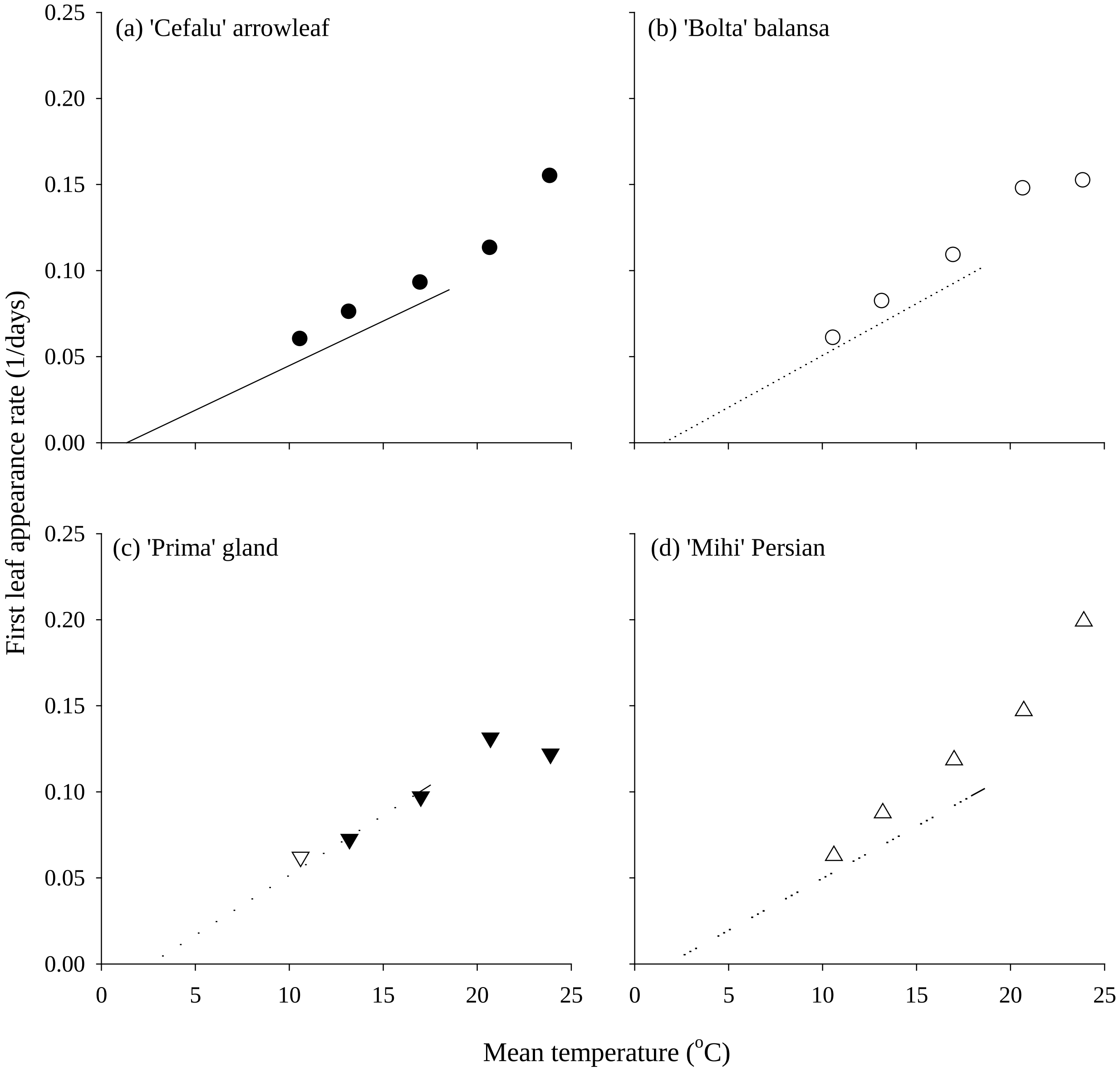


Figure 5.9 Calibrated (lines) and observed (symbols) first leaf appearance rate of (a) Cefalu' arrowleaf (●), (b) 'Bolta' balansa (○), (c) 'Prima' gland (▼) and (d) 'Mihi' Persian (△) clover sown in a controlled environment chamber at different mean soil (10 mm) temperatures at Lincoln University in 2012.

5.3.2.3 Fitted model using data from all locations

When the data from both the field and CEC experiments were pooled and reanalysed to fit a new model (Figure 5.10), the thermal time to first leaf appearance was estimated at 196 ^{o}C d for ‘Cefalu’ arrowleaf, 169 ^{o}C d for ‘Bolta’ balansa, 175 ^{o}C d for ‘Prima’ gland and 154 ^{o}C d for ‘Mihi’ Persian clovers all above a base temperature of 0 ^{o}C (Table 5.9).

Table 5.9 Base temperature (T_b) and thermal time (Tt) requirements for first leaf appearance of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Iversen 9 field, Lincoln University in 2010-2011 and in a controlled environment chamber at Lincoln University in 2012.

Species	T_b (^{o}C)	Tt (^{o}Cd)	R^2 (%)	[1]Tt (T_b=0 ^{o}C) (^{o}Cd)
‘Cefalu’ arrowleaf	1.5	176	93	196
‘Bolta’ balansa	2.6	139	89	169
‘Prima’ gland	2.6	142	95	175
‘Mihi’ Persian	3.7	117	91	154
Max. s.e.	0.27	7.4		4.5
95% c.i.	-1.5,7.5			

[1]Analysis assumes a base temperature of 0^{o}C. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

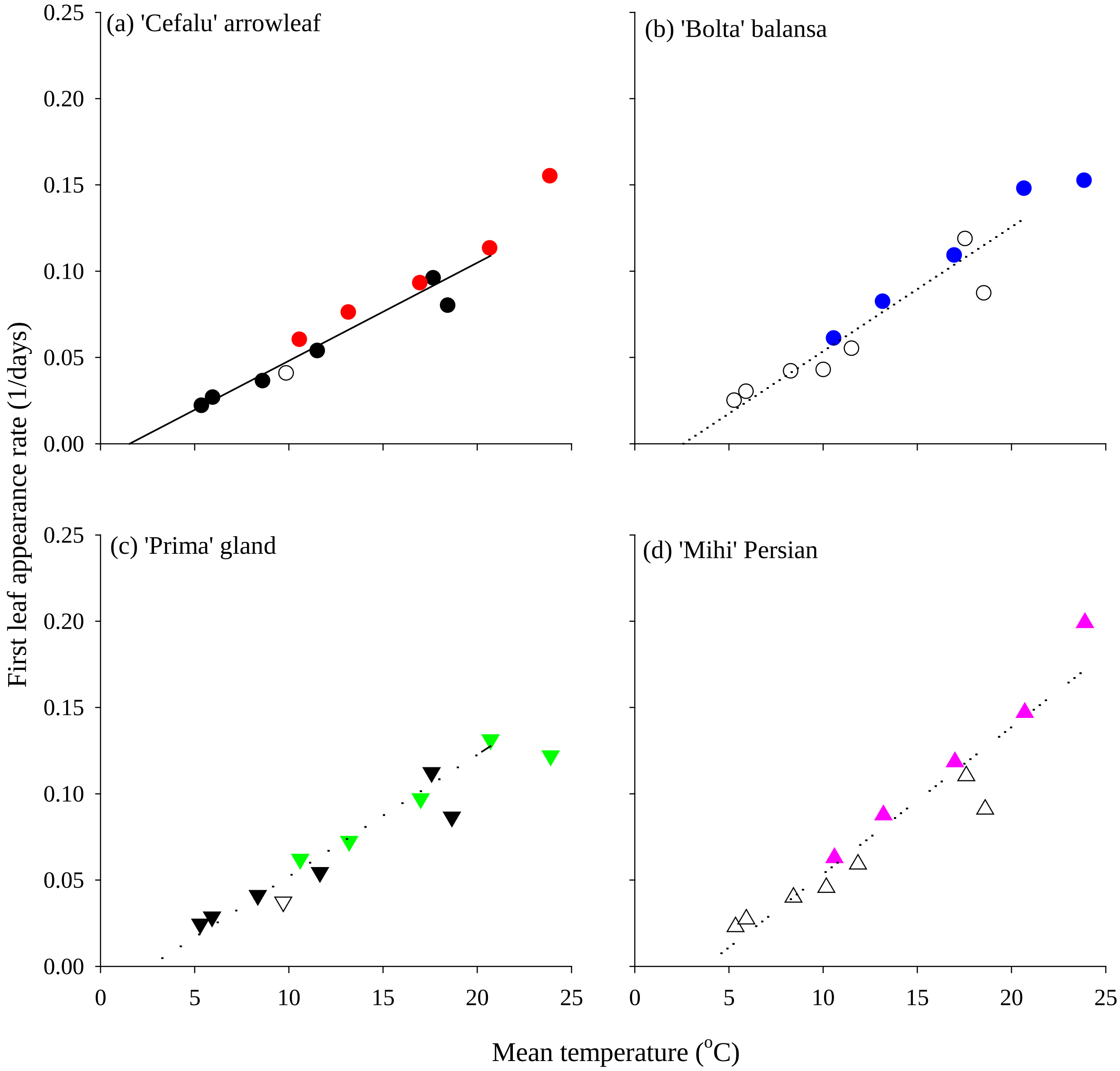


Figure 5.10 First leaf appearance rate of (a) ‘Cefalu’ arrowleaf (●,●), (b) ‘Bolta’ balansa (○,●), (c) ‘Prima’ gland (▼,▼) and (d)‘Mihi’ Persian (△,▲) clovers at different mean soil (10 mm) temperatures at Iversen 9 field, Lincoln University in 2010-2011 (black and white symbols) and a controlled environment chamber at Lincoln University in 2012 (coloured symbols).

5.3.3 Phyllochron

5.3.3.1 Controlled environment chamber (CEC)

The leaf appearance interval (days/leaf), or phyllochron, was greater than 7 days at 7.9 ^oC for each species, but decreased to ~3 days as air temperature increased to a mean of 25.4 ^oC (Figure 5.11a). Leaf appearance rate (leaves/day) was a linear function of mean air temperature from 7.9 to 25.4 ^oC (Figure 5.11b). Note, these plants were sown at an absolute photoperiod of 16.0 hours. The thermal time requirement to produce each successive leaf was 91 ^oC d for ‘Cefalu’ arrowleaf, 61 ^oC d for ‘Bolta’ balansa, 56 ^oC d for ‘Prima’ gland and 73 ^oC d for ‘Mihi’ Persian clover above a T_b of 0^oC (Table 5.10).

Table 5.10 Base temperature (T_b) and thermal time (Tt) requirements for phyllochron of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in a controlled environment chamber at Lincoln University in 2012.

Species	T_b (^oC)	Tt (^oC d)	R^2 (%)	[1]Tt (T_b=0^oC) (^oC d)
Cefalu' arrowleaf	0.7	87	93	91
Bolta' balansa	0.1	60	100	61
Prima' gland	1.1	52	98	56
Mihi' Persian	0.5	71	95	73
Maximum s.e.	0.46	12.1		4.2
95% c.i.	-4.7, 5.4			

[1]Analysis assumes a base temperature of 0^oC. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

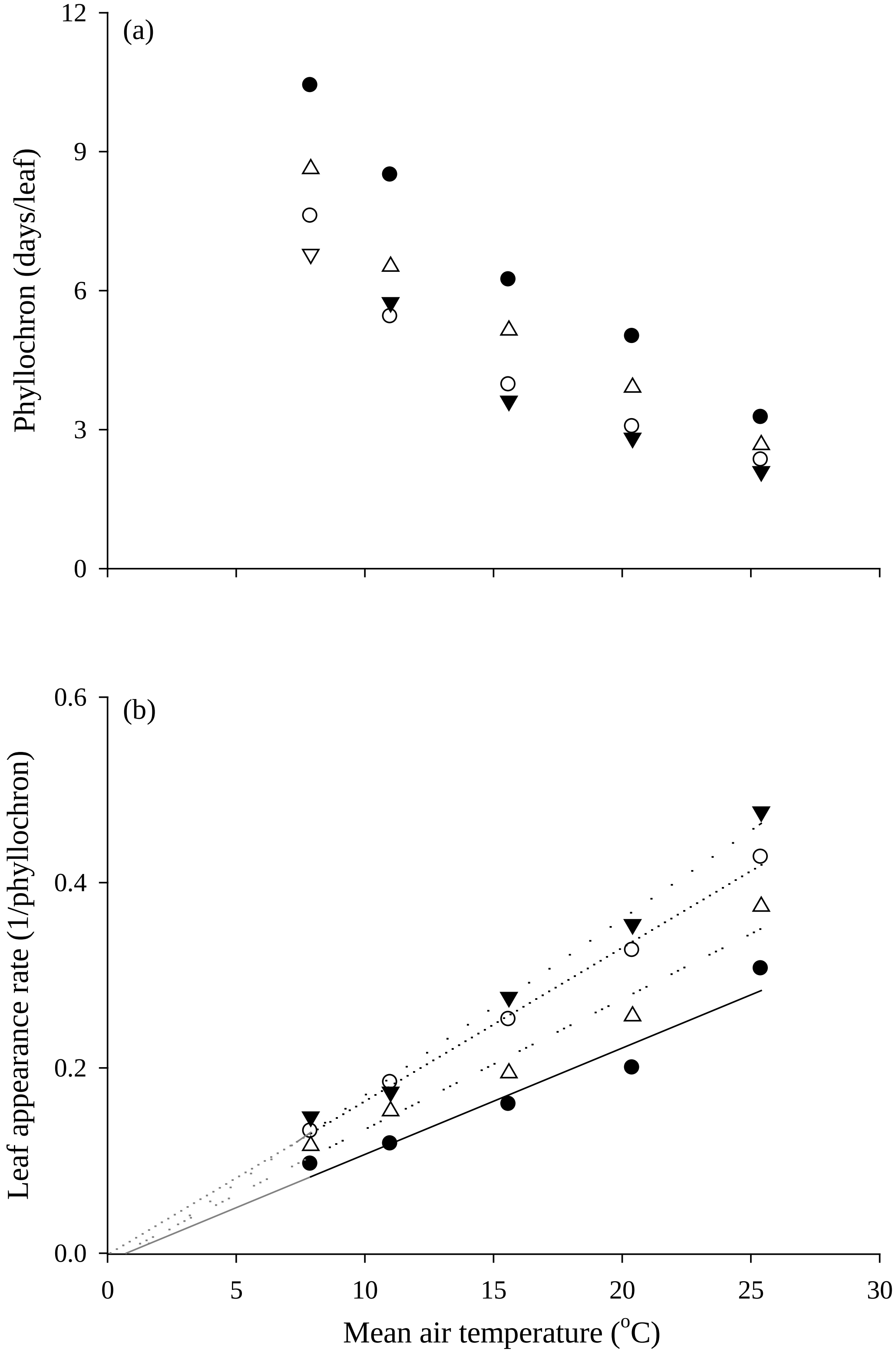


Figure 5.11 Phyllochron (days/leaf) (a) and leaf appearance rate (b) of ‘Cefalu’ arrowleaf (●), ‘Bolta’ balansa (○), ‘Prima’ gland (▼) and ‘Mihi’ Persian (△) clover at different mean air temperatures in a controlled environment chamber at Lincoln University in 2012. Gray lines are extrapolated lines.

5.3.3.2 Glasshouse nursery and off-site experiments

In all species, the appearance of leaves on the main stem increased linearly with thermal time accumulation (Figure 5.12). The slope of each linear relationship was used to calculate the phyllochron (^{o}C d/leaf).

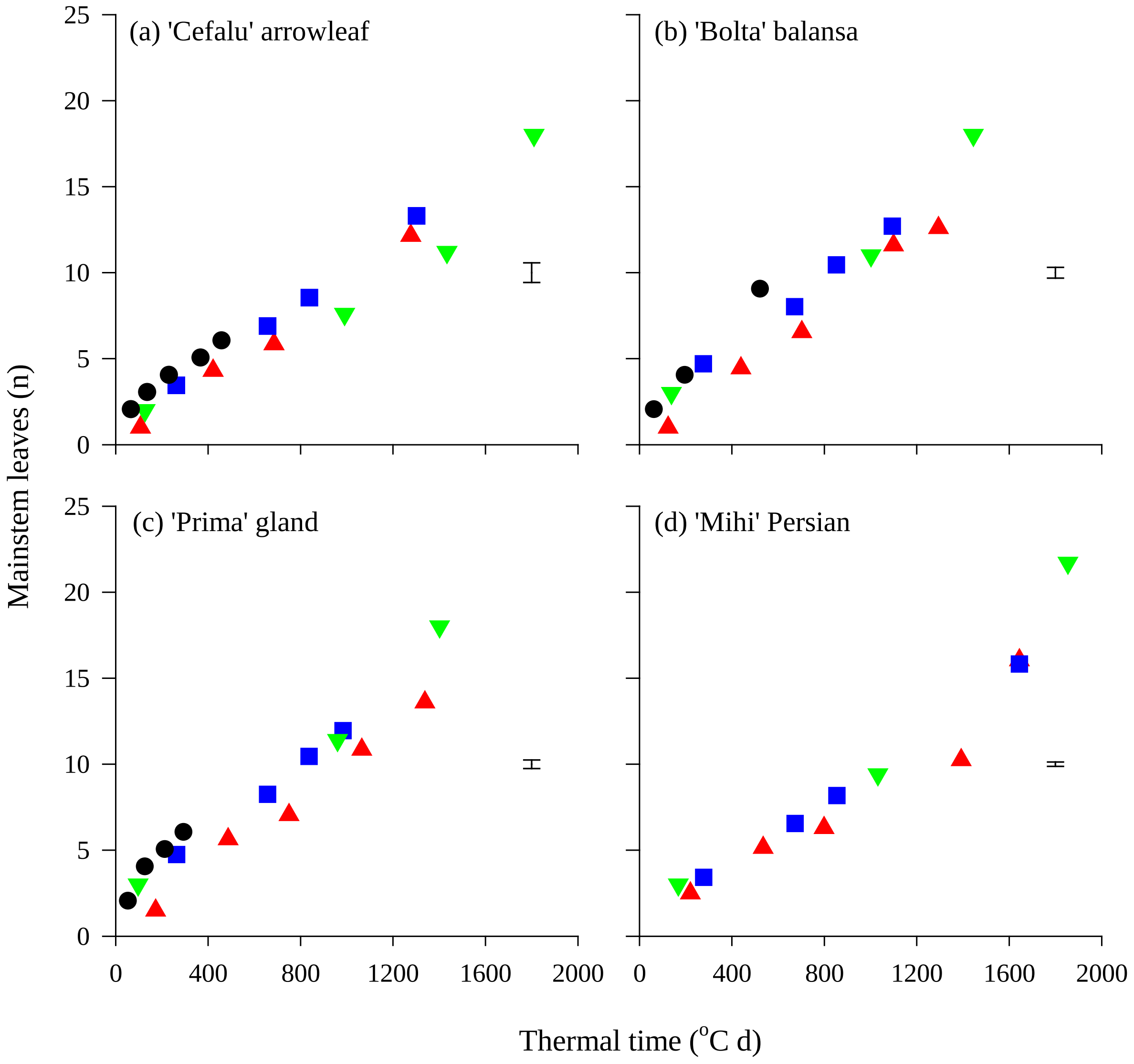


Figure 5.12 The number of leaves on the mainstem of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown at different locations (Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●)) versus accumulated thermal time. Note: Thermal time used air temperature (T_b = 0^{o}C) and calculated starting from first leaf appearance. Error bars represent maximum standard error for the final number of leaves.

5.3.3.2.1 Glasshouse nursery

The phyllochron for 'Bolta' balansa clover sown on 24 January 2012 was 65 (±0.4) ^{o}C d/leaf. For 'Cefalu' arrowleaf and 'Prima' gland clovers sown on 7 February 2012, their phyllochron were 103 (±6.2) ^{o}C d/leaf and 62 (±9.7) ^{o}C d/leaf, respectively.

5.3.3.2.2 Castle Hill, Canterbury

The phyllochron for plants sown on 11 March 2011 was 108 (±8.6) ^{o}C d/leaf for 'Cefalu' arrowleaf, 98 (±5.7) ^{o}C d/leaf for 'Bolta' balansa, 99 (±4.8) ^{o}C d/leaf for 'Prima' gland and 116 (±11.3) ^{o}C d/leaf for 'Mihi' Persian clover.

5.3.3.2.3 Lees Valley, Canterbury

The phyllochron for plants sown on 29 March 2011 was 105 (±3.5) ^{o}C d/leaf for 'Cefalu' arrowleaf, 101 (±6.1) ^{o}C d/leaf for 'Bolta' balansa, 100 (±4.8) ^{o}C d/leaf for 'Prima' gland and 109 (±3.9) ^{o}C d/leaf for 'Mihi' Persian clover.

5.3.3.2.4 Breach Oak, Blenheim

Plants sown on 15 April 2011 had a phyllochron of 111 (±20.1) ^{o}C d/leaf for 'Cefalu' arrowleaf, 89 (±13.0) ^{o}C d/leaf for 'Bolta' balansa, 89 (±10.3) ^{o}C d/leaf for 'Prima' gland and 90 (±17.8) ^{o}C d/leaf for 'Mihi' Persian clover.

5.3.3.3 Validation of the field experiment

The validation of the phyllochron model at different locations and sowing dates conformed with the predicted pattern that leaf appearance responded to the length and direction of photoperiod change (Figure 5.13). In all species, crops that were sown in the autumn had a longer phyllochron compared with summer sown when both emerged into a decreasing photoperiod. The phyllochron agreed with the predicted model with an RMSD of 3.8-24.6%. The glasshouse nursery plants that were sown in the summer into a 15.0 hour decreasing photoperiod followed the expected pattern for phyllochron. The phyllochron was similar for plants sown at Castle Hill (11th March 2011) and Lees Valley (29th March 2011). Species sown in the CEC with a constant 16.0 hour photoperiod had a longer phyllochron and their growth pattern tended to respond as though it was a decreasing photoperiod (Figure 5.13). This contrasted with those grown under field conditions into the same 16.0 hour length but with increasing photoperiod (Figure 3.12).

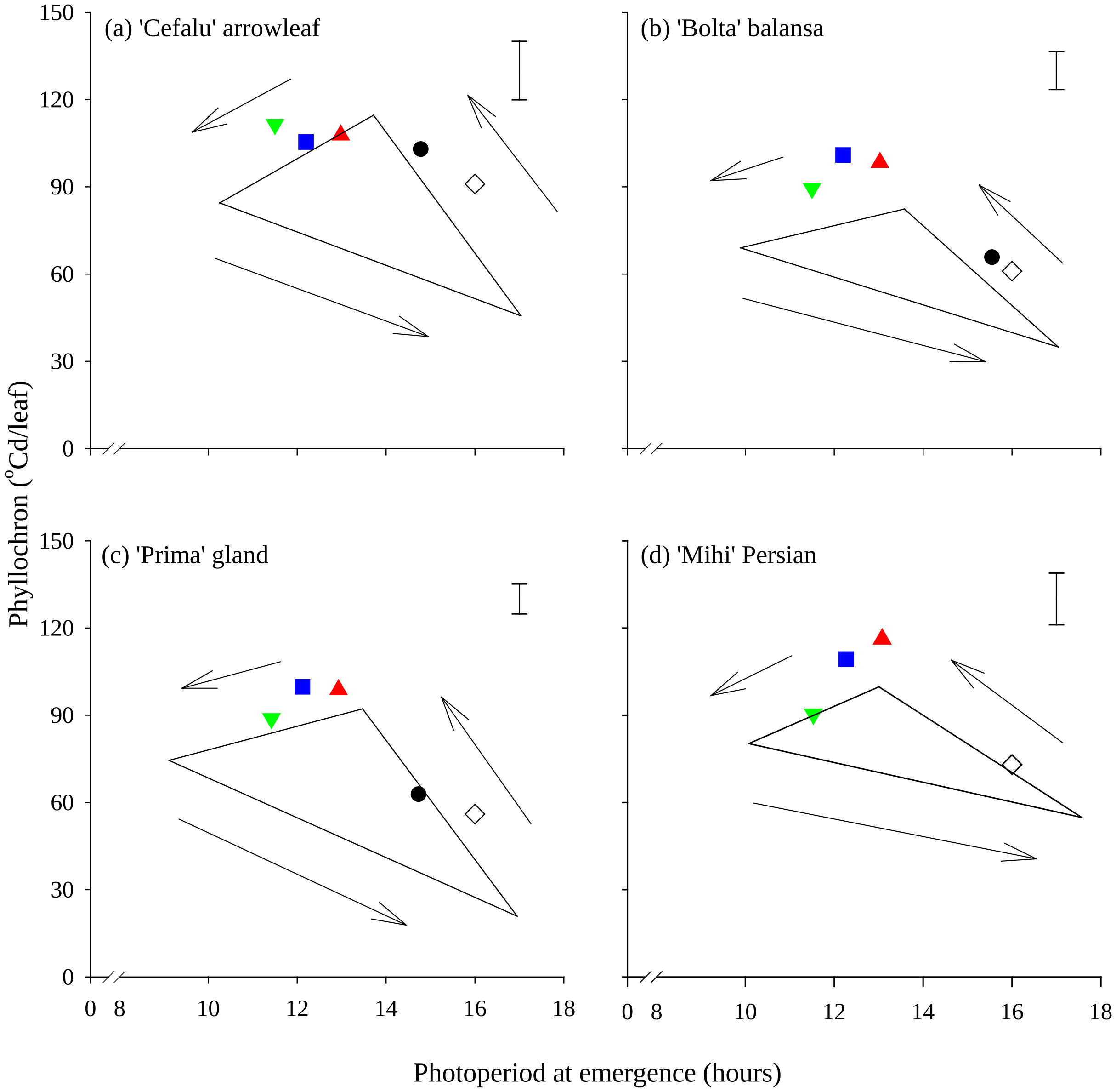


Figure 5.13 Calibrated (lines) and observed (symbols) phyllochron in response to photoperiod of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown at various locations. Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Controlled environment (◇).

5.3.3.4 Fitted model using data from all locations

The reanalysis of phyllochron in relation to photoperiod from the combined data from all locations (Figure 5.14) is summarized in Table 5.11. Autumn sown crops that grew into a decreasing photoperiod towards the winter had their phyllochron shorten by 8-12 ^{o}C d leaf^{-1} hour^{-1}. In contrast, summer sown crops that emerged into a decreasing photoperiod towards the autumn had a longer phyllochron by 13-18 ^{o}C d leaf^{-1} hour^{-1}.

Table 5.11 Coefficients of fitted relationship between phyllochron (^{o}Cd/leaf) and direction of photoperiod (hours) change in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clover using combination data from validation and calibration experiments.

Species	Direction of photoperiod	Relationship	R^2 (%)
‘Cefalu' arrowleaf	Increase	y = 143.3 - 5.73x	93
	Decrease into autumn	y = 346.9 – 17.16x	62
	Decrease into winter	y = 4.4 + 8.17x	65
‘Bolta' balansa	Increase	y = 116.2 - 4.77x	88
	Decrease into autumn	y = 269.1 - 13.43x	93
	Decrease into winter	y = -49.3 + 11.62x	47
‘Prima' gland	Increase	y = 136.8 - 6.84x	90
	Decrease into autumn	y = 333.0 – 18.05x	91
	Decrease into winter	y = -7.6 + 8.38x	79
‘Mihi' Persian	Increase	y = 114.5 - 3.39x	91
	Decrease into autumn	y = 282.0 - 13.18x	89
	Decrease into winter	y = -41.4 + 11.79x	82

R^2, coefficient of determination.

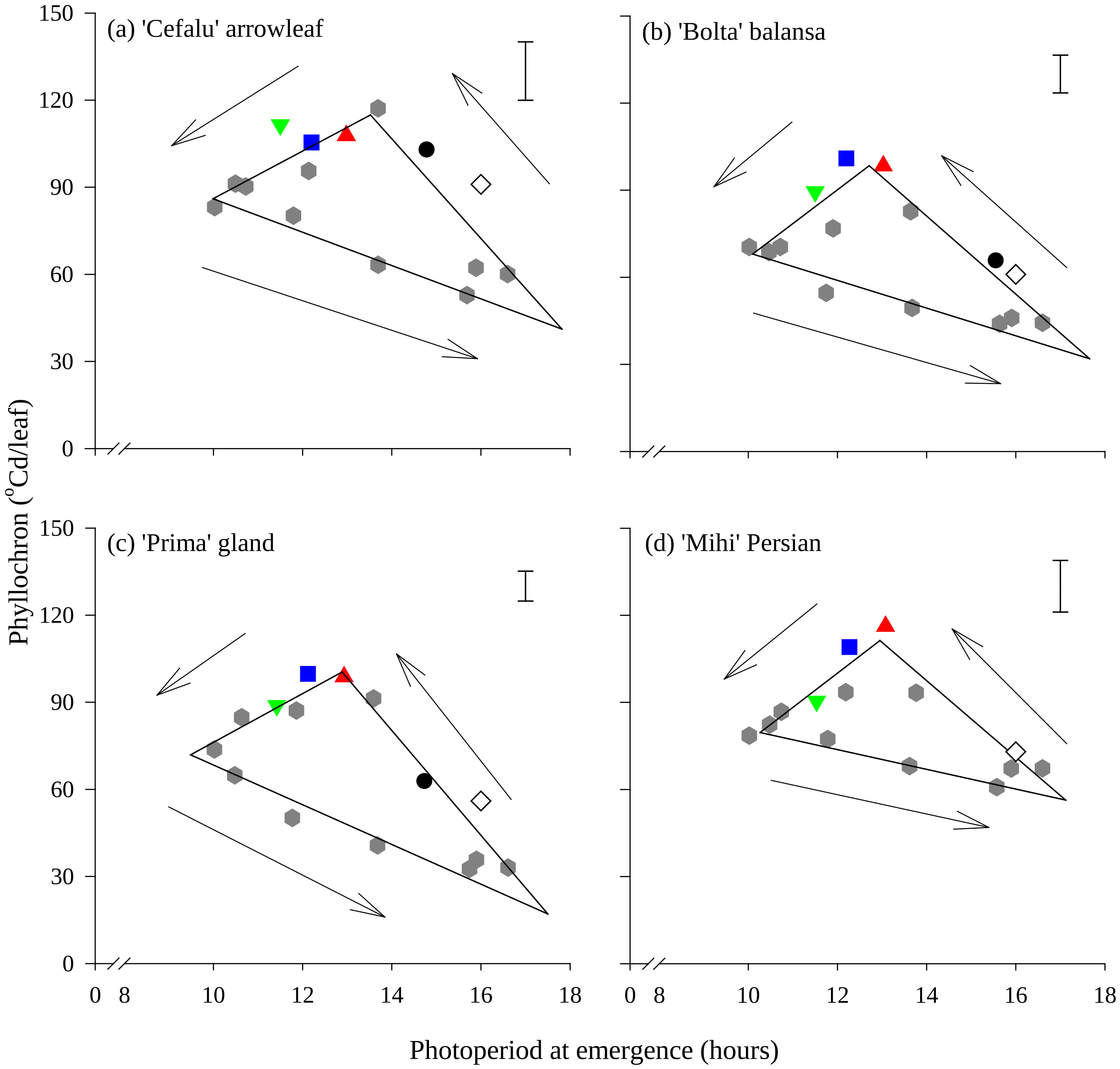


Figure 5.14 Phyllochron in response to photoperiod at emergence of (a) 'Cefalu' arrowleaf, (b) 'Bolta' blansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown at various locations. Iversen 9 field, Lincoln University (⬢), Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Controlled environment chamber (◇).

5.3.4 Appearance of axillary leaves

5.3.4.1 Controlled environment chamber (CEC)

The development of axillary leaves led to an exponential increase in the total number of leaves, whereas the number of leaves on the main stem continued to increase linearly with time (Figure 5.15). Above a T_b of 0 ^oC, it took 594 ^oC d for ‘Cefalu’ arrowleaf, 398 ^oC d for ‘Bolta’ balansa, 461 ^oC d for ‘Prima’ gland and 428 ^oC d for ‘Mihi’ Persian clover to produce their first axillary leaf (Table 5.12).

Table 5.12 Base temperature (T_b) and thermal time (Tt) requirements for first axillary leaf appearance of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in a controlled environment chamber at Lincoln University in 2012.

Species	T_b (^oC)	Tt (^oC d)	R^2 (%)	^{1}Tt (T_b=0^oC) (^oC d)
Cefalu' arrowleaf	-2.0	673	88	594
Bolta' balansa	-3.2	481	91	398
Prima' gland	-10.4	773	86	461
Mihi' Persian	-0.1	429	96	428
Maximum s.e.	0.51	80.7		18.9
95% c.i.	-19.0, 3.0			

[1] Analysis assumes a base temperature of 0^{o}C. R^2, coefficient of determination; s.e., standard error; c.i., confidence interval.

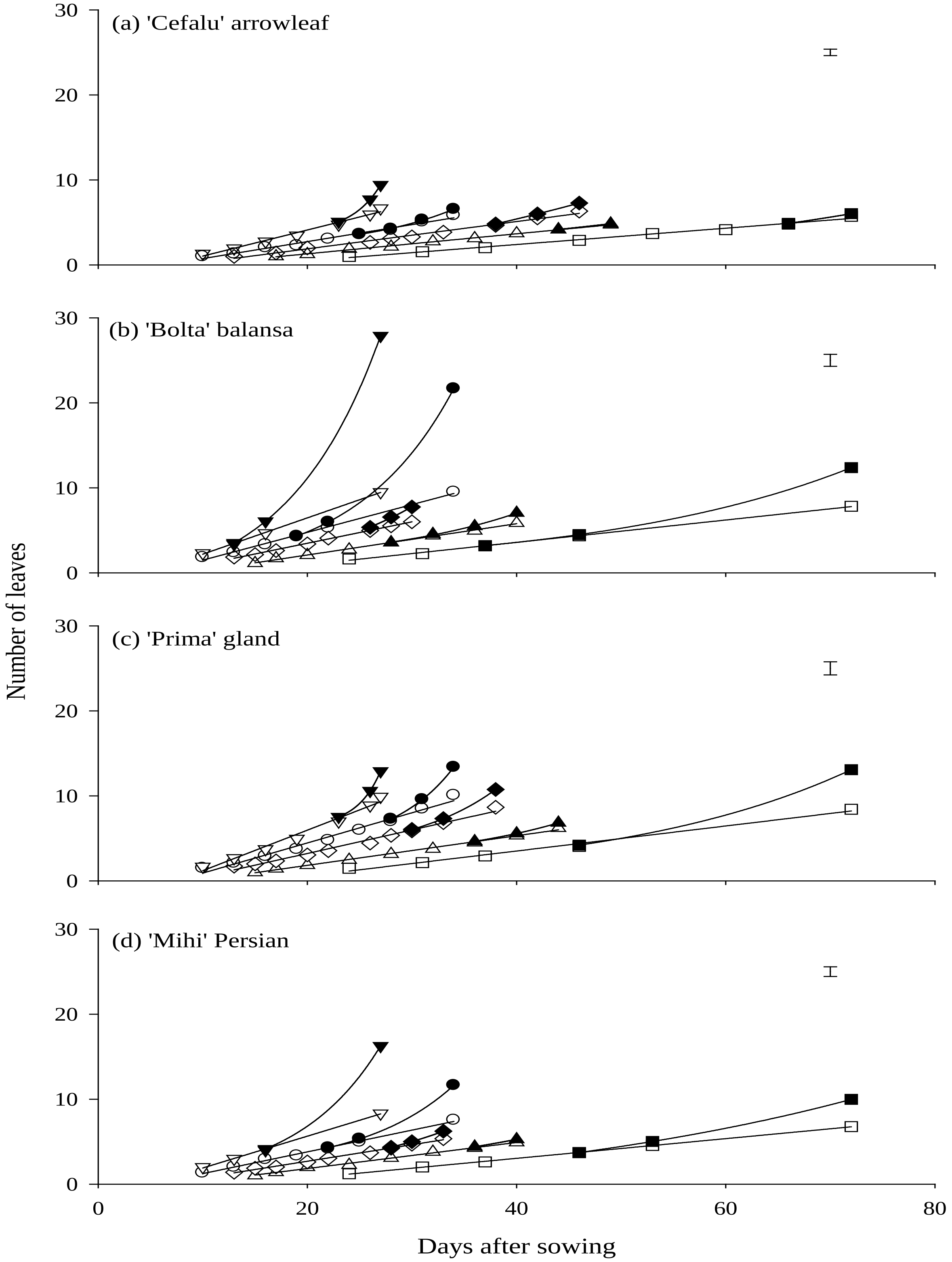


Figure 5.15 Number of total (closed symbols) and main stem (open symbol) leaves of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clover plotted against days after sowing at mean air temperature of 7.9 (■), 11(▲), 15.6 (◆), 20.4 (●) and 25.4 (▼) ^{o}C in a controlled environment chamber at Lincoln University in 2012. Error bars represent the maximum standard error for the final total leaf number.

5.3.4.2 Glasshouse nursery

The thermal time requirement for first axillary leaf appearance was 672 (±13.7) ^{o}C d for ‘Cefalu’ arrowleaf, 354 (±19.3) ^{o}C d for ‘Bolta’ balansa and 405 (±8.8) ^{o}C d for ‘Prima’ gland clover.

5.3.4.3 Off-site locations

The thermal time to first axillary leaf appearance for annual clovers sown in Castle Hill, Lees Valley and Breach Oak were summarized in Table 5.13.

Table 5.13 Thermal time (^{o}C d) from sowing to first axillary leaf appearance of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim in 2011.

Species	Castle Hill, Canterbury	Lees Valley, Canterbury	Breach Oak, Blenheim
‘Cefalu’ arrowleaf	833	768	911
‘Bolta’ balansa	696	586	783
‘Prima’ gland	589	563	739
‘Mihi’ Persian	682	693	754
Maximum s.e.	33.2	16.8	63.2

Thermal time quantified based on air temperature ($T_b = 0^{o}C$). s.e. standard error.

5.3.4.4 Validation of the field experiment

The time to axillary leaf production for annual clovers sown to validate the original development model showed strong agreement with the observed data (Figure 5.16) with an RMSD less than 14%.

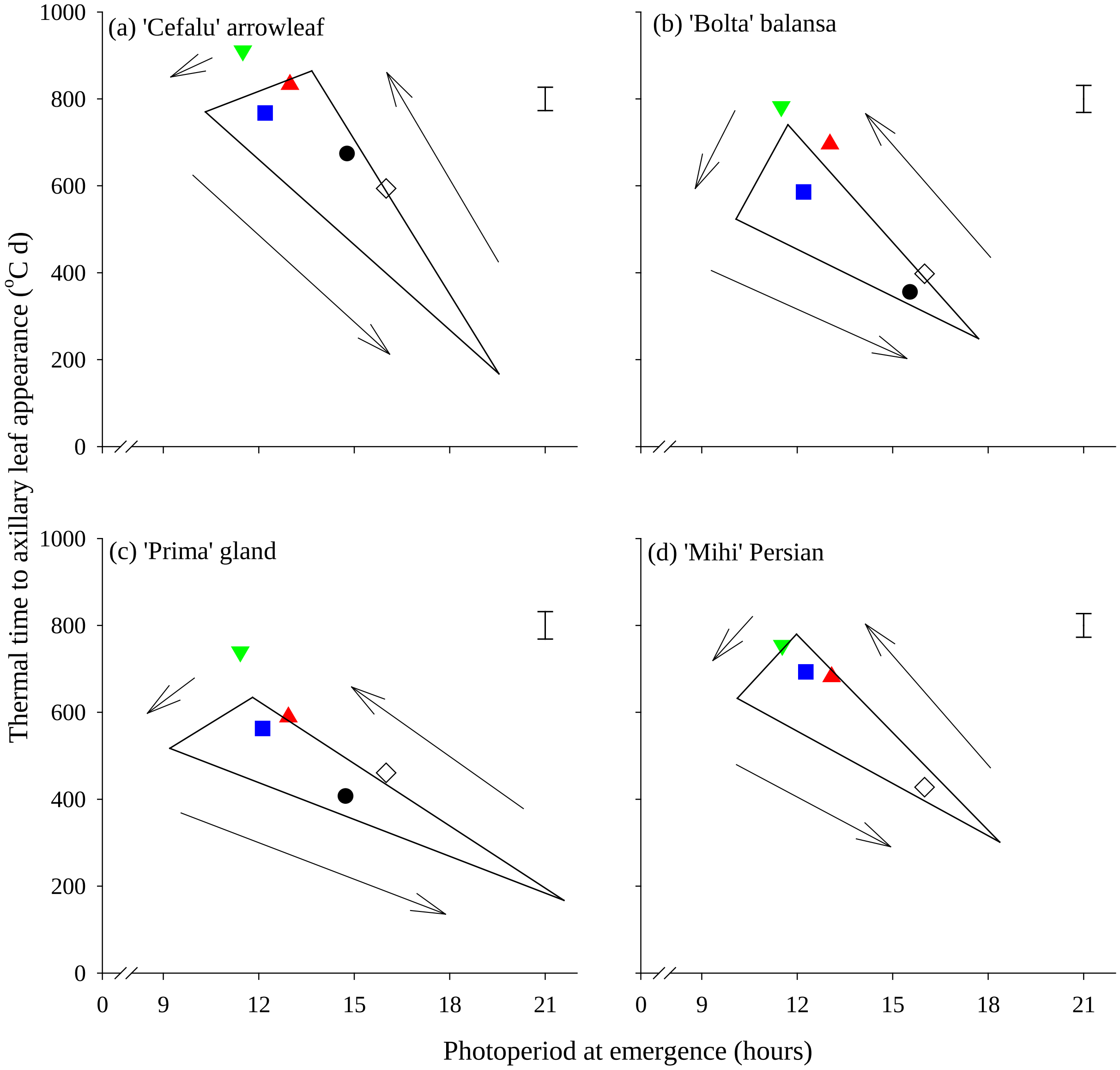


Figure 5.16 Calibrated (lines) and observed (symbols) time from sowing to axillary leaf appearance in response to photoperiod of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown at various locations. Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Controlled environment chamber (◇).

5.3.4.5 Fitted model using data from all locations

The final model from reanalysis of data from all locations (Figure 5.17) did not improve the relationship between thermal time to axillary leaf and photoperiod change. The coefficient of determination (R^2) was lower (Table 5.14) than from the calibration model (Table 3.11).

Table 5.14 Coefficients of fitted relationship between time to axillary leaf appearance (^oC d) and direction of photoperiod (hours) change in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover using combination data from validation and calibration experiments.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	y = 144 -65.3x	91
	Decrease into autumn	y = 2363 – 111.3x	61
	Decrease into winter	y = 568 + 21.2x	5
'Bolta' balansa	Increase	y = 888 - 36.1x	86
	Decrease into autumn	y = 1635 – 78.5x	78
	Decrease into winter	y = -972 + 148.7x	85
'Prima' gland	Increase	y = 777 - 28.3x	92
	Decrease into autumn	y = 1127 – 43.8x	76
	Decrease into winter	y = -159 + 71.7x	26
'Mihi' Persian	Increase	y = 1038 - 40.1x	93
	Decrease into autumn	y = 1618 – 72.1x	86
	Decrease into winter	y = -186 + 81.0x	90

R^2, coefficient of determination.

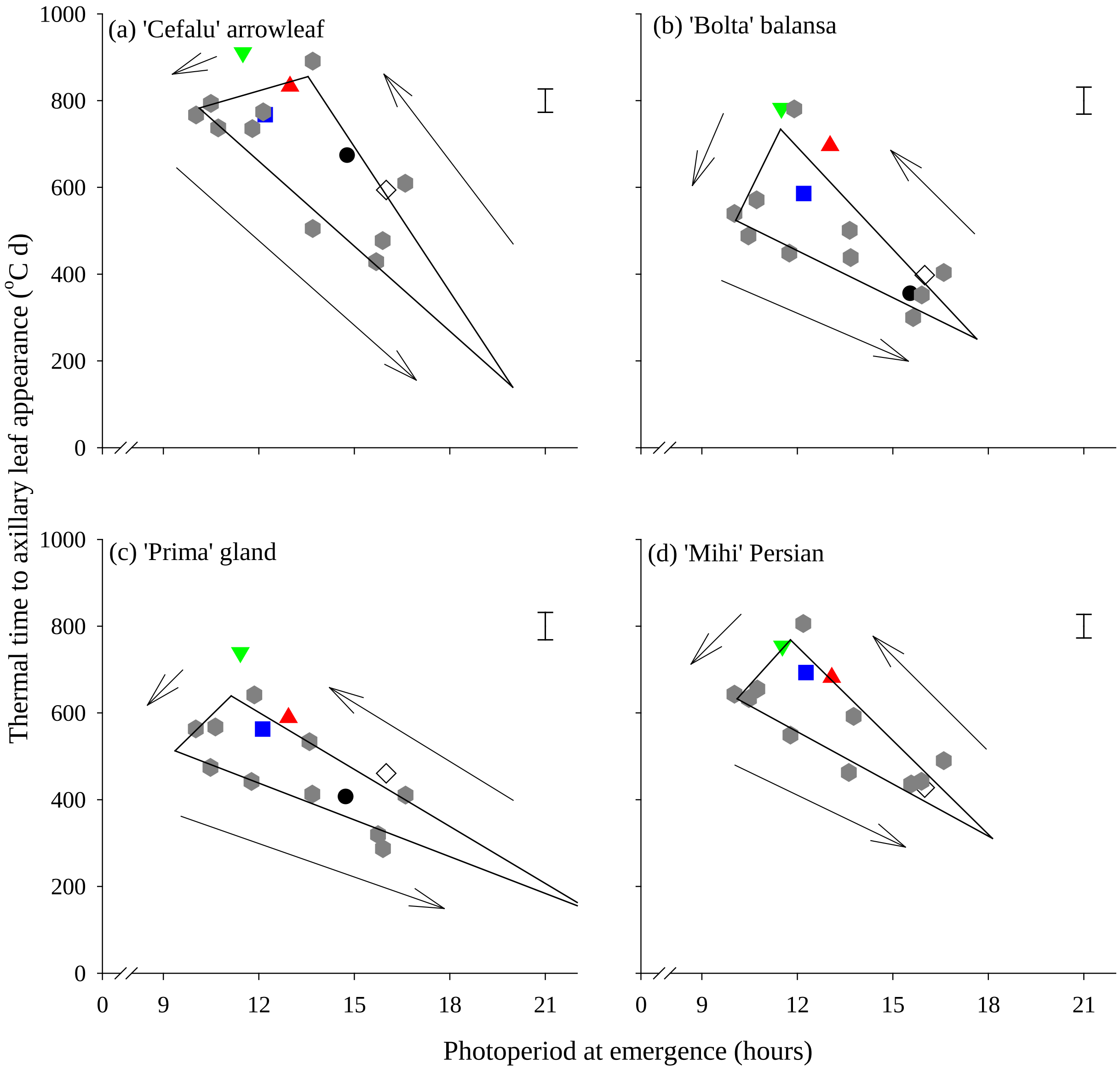


Figure 5.17 Thermal time from sowing to axillary leaf appearance in response to photoperiod at emergence of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ blansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown at different locations. Iversen 9 field, Lincoln University (⬢), Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Controlled environment chamber (◇).

5.3.5 Time to flowering

5.3.5.1 Glass house nursery

‘Cefalu’ arrowleaf clover sown on 7th February 2012 flowered on 19th August 2012 after 185 days and 1794 °C d from emergence (Table 5.15). ‘Bolta’ balansa clover sown on 24th January 2012 flowered on 2nd April 2012. The plants took 62 days and 820 °C d from emergence to flower. ‘Prima’ gland clover sown on 7th February 2012 flowered on 1st May 2012 after 74 days and 865 °C d from emergence. ‘Mihi’ Persian clovers sown on 20th December 2011 and 19th January 2012 took 78 and 106 days to flower respectively. The thermal time requirement to flower for ‘Mihi’ Persian was 1485 °C d for December sown plants and 1769 °C d for January sown plants. However, for both sowing dates, flowering only occurred in 25% of the plant population. Therefore, time to flowering for ‘Mihi’ Persian clovers sown on these two dates was quantified based on only the 25% bud visible phase within the population rather than the usual 50% (Section 5.2.2.3.4).

Table 5.15 Time from emergence to flowering quantified in days and thermal time (°C d) for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown at different dates in glasshouse nursery, Lincoln University, Canterbury, New Zealand.

Species	Sowing date	Flower date	Days to flower	Thermal time to flower
‘Cefalu’ arrowleaf	7/2/2012	19/8/2012	185	1794
‘Bolta’ balansa	24/1/2012	2/4/2012	62	820
‘Prima’ gland	7/2/2012	1/5/2012	74	865
‘Mihi’ Persian	20/12/2011	11/3/2012	78	1485
	19/1/2012	8/5/2012	106	1769
Maximum s.e.			4.9	77.6

Thermal time quantified based on air temperature (T_b = 0°C). s.e. standard error.

5.3.5.2 Experiments in Iversen 9 field, Lincoln University in year 2011

5.3.5.2.1 Additional sowing for ‘Mihi’ Persian clover

‘Mihi’ Persian clover sown on 17th October 2011 emerged on 27th October 2011. The plants flowered on 29th December 2011 after 63 days and 902 (±56.4) ^{o}C d (air temperature, T_b = 0^{o}C).

5.3.5.2.2 First year regeneration of annual clovers sown in 2010

5.3.5.2.2.1 ‘Cefalu’ arrowleaf clover

Seedlings emerged between 10th - 20th March 2011 and flowered between 11th – 15th October 2011 (Table 5.16). The plants took 212 days and 1938 ^{o}C d to flower.

5.3.5.2.2.2 ‘Bolta’ balansa clover

The earliest seedlings emerged between 2nd - 13th January 2011 and flowered between 12th – 23rd February 2011 (Table 5.16). The plants required 41 days and 582 ^{o}C d to flower.

5.3.5.2.2.3 ‘Prima’ gland clover

The earliest seedlings emerged in the 3rd week of January 2011. All plants took the same amount of time (44 days and 574 ^{o}C d) to flower in the 1st week of March 2011 (Table 5.16).

5.3.5.2.2.4 ‘Mihi’ Persian clover

All seedlings emerged on the same date on 23rd March 2011 and flowered between 20th - 22nd November 2011 (Table 5.16). These plants required 243 days and 2326 ^{o}C d to flower.

Table 5.16 Dates of emergence and flowering for first year regeneration of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Species	2010 Sowing date	[1]Maturity date	2011 Emergence date	2011 Flowering date
‘Cefalu’ arowleaf	26 Feb	10 Jan 11	10 Mar	11 Oct
	24 Mar	11 Jan 11	21 Mar	15 Oct
	19 Apr	11 Jan 11	12 Mar	15 Oct
	8 Jul	17 Jan 11	20 Mar	15 Oct
‘Bolta’ balansa	26 Feb	25 Nov 10	2 Jan	12 Feb
	24 Mar	14 Dec 10	3 Jan	13 Feb
	19 Apr	16 Dec 10	2 Jan	12 Feb
	8 Jul	2 Jan 11	13 Jan	23 Feb
‘Prima’ gland	26 Feb	25 Nov 10	17 Jan	2 Mar
	24 Mar	8 Dec 10	16 Jan	1 Mar
	19 Apr	16 Dec 10	19 Jan	4 Mar
	8 Jul	16 Dec 10	19 Jan	4 Mar
‘Mihi’ Persian	26 Feb	18 Jan 11	23 Mar	20 Nov
	24 Mar	18 Jan 11	23 Mar	22 Nov
	19 Apr	25 Jan 11	23 Mar	20 Nov
	8 Jul	25 Jan 11	23 Mar	20 Nov

[1]Refers to the time of harvest maturity.

5.3.5.3 Off-site locations

The time to flowering quantified in days and thermal time for plants sown in Castle Hill, Lees Valley and Breach Oak were summarized in Table 5.17 and Table 5.18.

Table 5.17 Number of days to flowering for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim in 2011.

Species	Castle Hill, Canterbury	Lees Valley, Canterbury	Breach Oak, Blenheim
‘Cefalu’ arrowleaf	228	200	176
‘Bolta’ balansa	211	185	157
‘Prima’ gland	198	171	141
‘Mihi’ Persian	261	234	215
Maximum s.e.	1.5	1.3	20.0

s.e. standard error.

Table 5.18 Thermal time to flowering (^{o}C d) for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim in 2011.

Species	Castle Hill, Canterbury	Lees Valley, Canterbury	Breach Oak, Blenheim
‘Cefalu’ arrowleaf	1409	1419	1663
‘Bolta’ balansa	1252	1270	1471
‘Prima’ gland	1143	1134	1307
‘Mihi’ Persian	1769	1800	2184
Maximum s.e.	19.3	11.9	197.4

Thermal time quantified based on air temperature ($T_b = 0^{o}C$). s.e. standard error.

Note, for plants in Breach Oak, Blenheim, there was a large variation between the two replicates where those that were sown on the west side of the valley received more sun compared with the east side which was more shaded. Hence, the plants on the west side were much bigger and tended to flower earlier chronologically than those on the east (Plate 15). However, the temperature sensors indicated a similar thermal time requirement to flowering.

Plate 15 The size of ‘Prima’ gland clovers on the (a) east versus (b) west side of the valley in Breach Oak, Blenheim on 24th September 2011.

5.3.5.4 Validation of the field experiment

There was agreement between the time to flowering observed in validated experimental sites and those estimated from the calibration data with an RMSD of 6.4 – 17.4%. The observed data in validation experiments conformed with the calibration model that the time to flowering of annual clovers was related to the direction and length of photoperiod at first trifoliate leaf (Figure 5.18). ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers sown in the Castle Hill and LeesValley produced their first trifoliate leaf at the same photoperiod duration and direction and also accumulated the same amount of thermal time to flower (Figure 5.18a,b,c). In most of the locations, flowering time was predicted accurately by the model. In ‘Bolta’ balansa clover, the reanalysis of flowering data from Monks (2009) across six sowing dates matched reasonably well with the fitted model, with the exception of a January sowing date when emergence was delayed until February (Figure 5.18b). There was also an over-estimation in the duration to flowering for ‘Mihi’ Persian clovers sown at Castle Hill and Lees Valley (Figure 5.18d). For plants sown in the glasshouse nursery, flowering ocurred earlier for ‘Bolta’ balansa (Figure 5.18b) and much later for ‘Cefalu’ arrowleaf clover than expected from the field experiment (Figure 5.18a).

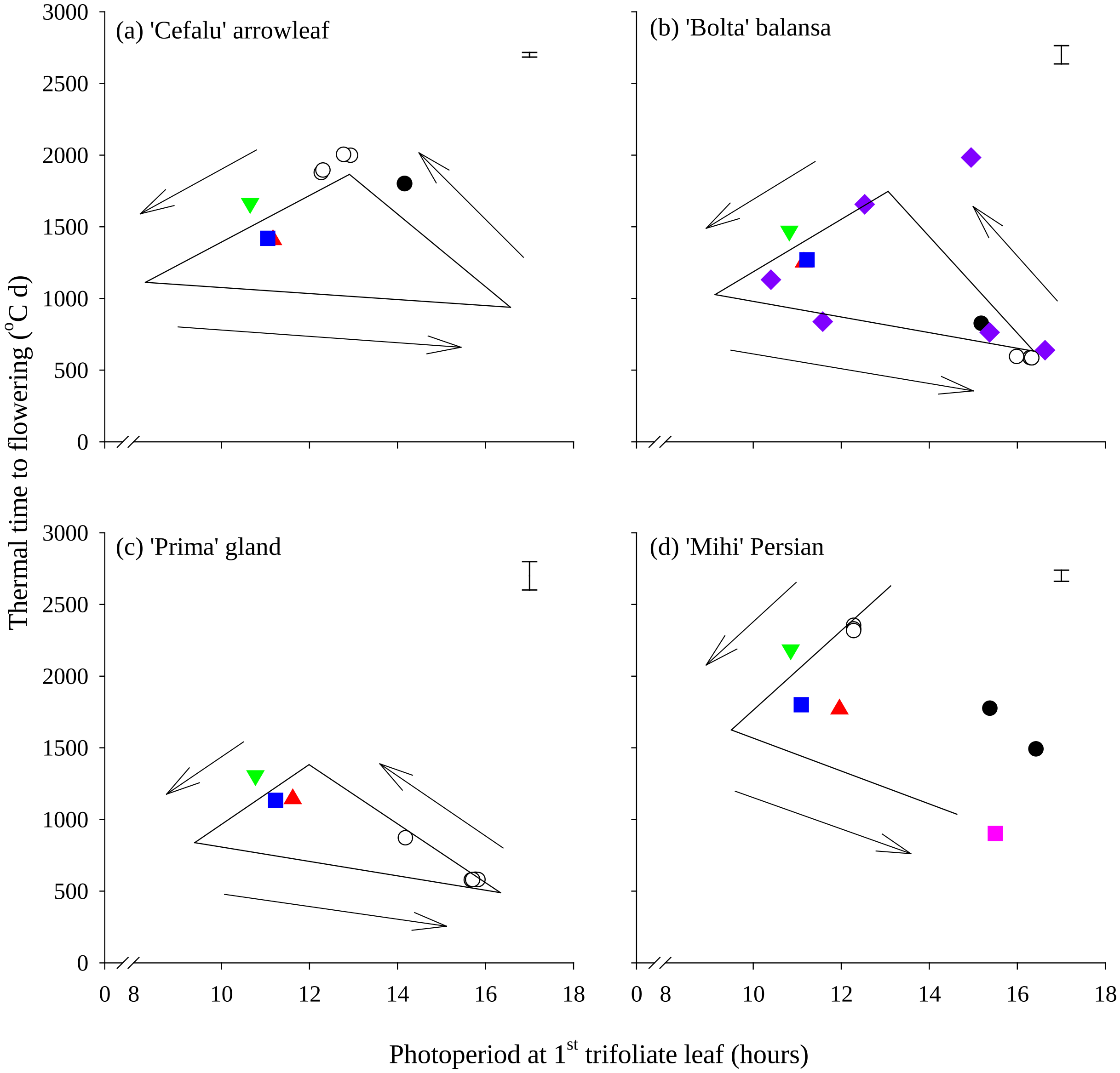


Figure 5.18 Calibrated (lines) and observed (symbols) thermal time to flowering in response to photoperiod of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown at various locations. Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Iversen 9 regeneration plots (○), Iversen 9 additional sowing (■), Monks *et al*. 2010 (◆).

5.3.5.5 Fitted model using data from all locations

Following validation of the flowering model, the flowering data from all experiments were combined and the models were re-fitted to the larger data sets (Figure 5.19). In all species, the new fitted model did not improve the description of the flowering time (R^2 = 52-99%) (Table 5.19) compared with the calibrated model (R^2 = 82-100%) (Table 4.5). For 'Mihi' Persian clover, flowering data from experiments conducted in the glasshouse nursery, Lincoln University generated a function that could be used to predict time to flowering for summer sown plants (Figure 5.19d).

Table 5.19 Coefficients of fitted relationship between time to flowering (^{o}C d) and direction of photoperiod (hours) change at first trifoliate leaf stage in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover using combination data from validated and calibrated experiments.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	y = 1285 -20.9x	43
	Decrease into autumn	y = 5508 – 274.3x	86
	Decrease into winter	y = -734 + 208.1x	75
'Bolta' balansa	Increase	y = 1525 - 54.6x	93
	Decrease into autumn	y = 6234 – 347.0x	96
	Decrease into winter	y = -588 + 175.9x	65
'Prima' gland	Increase	y = 1309 - 50.1x	92
	Decrease into autumn	y = 3800 - 204.0x	96
	Decrease into winter	y = 127 + 94.0x	
'Mihi' Persian	Increase	y = 2779 - 120.1x	98
	Decrease into autumn	y = 7254 - 354.7x	99
	Decrease into winter	y = -706 + 243.7x	52

R^2, coefficient of determination.

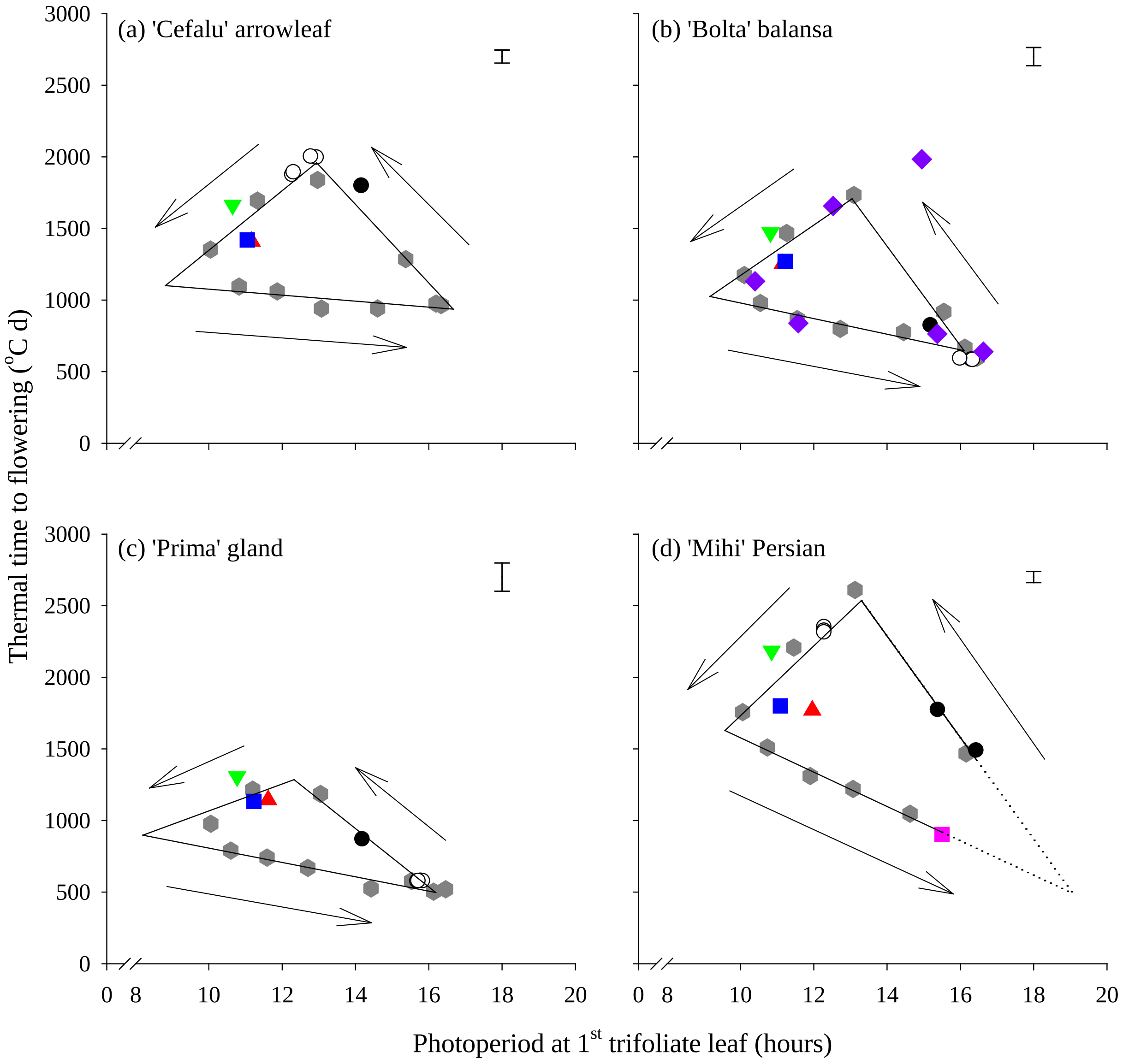


Figure 5.19 Thermal time from emergence to flowering in response to photoperiod at first trifoliate leaf of (a) 'Cefalu' arrowleaf, (b) 'Bolta' blansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown at various locations. Iversen 9 field, Lincoln University (⬢), Castle Hill, Canterbury (▲), Lees Valley, Canterbury (■), Breach Oak, Blenheim (▼), Glass house, nursery, Lincoln University (●), Iversen 9 regeneration plots (○), Iversen 9 additional sowing (■), Monks *et al*. 2010 (◆). Dotted lines are extrapolated lines.

5.4 Discussion

5.4.1 Emergence

In all species, the emergence rate as a function of mean soil temperature in CEC agreed with the germination rate data presented in Figure 3.6. For ‘Bolta’ balansa and ‘Prima’ gland clovers, the emergence rate declined at temperatures above 20 ^{o}C which conformed to their germination model. The exception was for ‘Cefalu’ arrowleaf clover, where the emergence rate increased at 23.9 ^{o}C which was unexpected based on the cardinal temperature model developed for germination. This discrepancy suggests that the optimum temperature of 20^{o}C for germination may be unsuitable to describe the optimum temperature for emergence of ‘Cefalu’ arrrowleaf clover. This lack of compatibility may be justified by the differences in methods of conducting the germination and emergence experiment. In the germination experiment, seeds were exposed to a constant temperature inside an incubator (Section 3.2.1.1) whereas for the emergence experiment, seeds experienced fluctuating temperatures in the CEC (Section 5.2.1.1). This discrepancy could also be due to physical errors in the recording of the temperatures in the CEC. The temperature setting of the CEC was set at 20/30 ^{o}C with an 8 h/8 h with 4 h transitions between day and night (Section 5.2.1.2). The frequency of irrigating twice a day cooled the soil temperature and produced a mean daily soil temperature of 23.9 ^{o}C. It is unclear how the seeds and seedlings would be affected by this temperature change. Thus, future experiments for germination should be carried out in a fluctuating 20/30 ^{o}C to confirm the cardinal temperatures.

The calibration model from field data accurately predicted the emergence rate as a function of mean soil temperature for both ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers sown in the CEC (Figure 5.7a,c). The CEC results suggest the linear relationship should be extended to 20 ^{o}C to correspond with the observed optimum temperature (Figure 3.6). The emergence of ‘Bolta’ balansa and ‘Mihi’ Persian clovers in the CEC was determined by a single linear relationship due to limited temperatures below 11 ^{o}C. For both species, the rate of seedling emergence at temperatures above 11 ^{o}C was over estimated (Figure 5.7b,d). However, these models were fitted using only 2 - 3 data points (R^2 = 27- 43%) (Figure 3.9b, Table 3.5) and limited data points (due to limited temperature range) may be insufficient to accurately determine the time to emergence. The accuracy of this model could be improved by using more temperatures beyond 11 ^{o}C.

The combined data model from Iversen 9 field and CEC experiments provided an improved description of the rate of emergence (Figure 5.7 and Figure 5.8). The percentage of variance in the combined data was higher (R^2 = 43-83%) (Table 5.7) compared with the initial calibration model (R^2 = 27-82%) (Appendix 2). Hence, the combined data can be considered the most accurate description of seedling emergence, but it now remains to be validated independently.

5.4.2 First leaf appearance

The rate of first leaf appearance as a function of mean soil temperature in the CEC followed the same pattern as seedling emergence in the field (Figure 5.7). The results from the validation experiment were more consistent than those estimated from the field (Figure 3.10), i.e. the rate of first leaf appearance increased linearly with mean soil temperature up to an optimum. Except for 'Prima' gland clover, the appearance of first leaf in the CEC was faster than reported from the field experiment. The field derived model under estimated the rate of first leaf appearance (Figure 5.9). It is likely this delayed seedling emergence in the field was due to the sowing date on 9th November 2010 (mean soil temperature: 18.5 ^{o}C) that experienced water stress which crusted the soil surface (Figure 3.9). This delayed field emergence and affected the timing of first leaf appearance. Therefore, it is suggested that this sowing date could be excluded from the analysis. Alternatively, this problem could be minimized by incorporating the hydrothermal time approach. The concept of hydrothermal time enables timing of seed germination at any given moisture condition and temperature to be estimated (Bradford, 1995). This information can be used to predict time of seedling emergence (Bradford, 2002) in the field. The incubator and CEC are the preferred environments for quantifying emergence because they minimize undesirable variations in the field conditions (eg. water stress). Thus, the results from there are considered the most accurate. However, the CEC's do not grow plants in the field under normal environmental conditions and are therefore less practical for examining the effects of photoperiod on plant development.

The new model fitted using the extended data sets from both field and controlled environment experiments improved the relationship between the rate of first leaf production and temperature. The percentage of variance in the combined data explained by the model was

89-95% (Table 5.9), and therefore considered to be the most accurate predictor for first leaf emergence.

5.4.3 Phyllochron

The calibration model provided an accurate prediction of leaf appearance rate of these annual clovers and showed the phyllochron was dependent on the photoperiod at the time and location of emergence. This was confirmed by the validation experiments. For example, the observed phyllochron for species sown between January and April into a decreasing photoperiod (Figure 5.13) verified the calibration experiments in Iversen 9 field, Lincoln University (Figure 3.12). Except for 'Mihi' Persian clover, the phyllochron for plants sown in the CEC were longer than those field estimated (Figure 5.13). The reason for this discrepancy is unclear, but the absence of a direction of photoperiod change in the CEC may have influenced leaf appearance. This highlights the trade-off between the precision of results obtained in a CEC and the accuracy with which they reflect the dynamics of a field environment. The advantages of growing plants in a CEC are that it could extend the range of temperatures above the optimum, control growth parameters and minimize variations in the field due to abiotic stresses and biotic interactions. The limitation of using a CEC was the unnatural environmental conditions for plant development. For example, the absence of photoperiod change in the CEC made it less practical for quantifying leaf appearance and flowering as these developments are influenced by photoperiod. To account for this, the next set of experiments in the CEC should be carried out using a constant temperature but with different daylengths. For 'Bolta' balansa grown in the Lees Valley, the phyllochron was much higher than estimated (RMSD = 30.4%) because of large variation (CV = 16.9%) among the replicates.

The combination data model from all locations (Figure 5.14) did not satisfactorily describe a strong relationship between phyllochron and photoperiod (Table 5.11). This method caused large distance between the data points from calibrated experiments and the fitted line (Figure 5.14b,d), thus reduced the variation percentage (R^2) of the linear relationship. The calibration model from Iversen 9 field, Lincoln University (Figure 3.12) was thus most appropriate for predicting the phyllochron of these annual clovers. The model validated at five different locations and photoperiods accounted for 3.8 – 24.6% of root mean square deviation (RMSD) of the predicted mean (Section 5.3.3.3). The data could be improved if leaf appearance was inspected at weekly intervals (as done in the calibration experiment) but this was physically impossible at the remote on-farm sites. Quantification of the phyllochron in response to decreasing photoperiod towards the autumn still requires more data points to accurately define the relationship.

5.4.4 Appearance of axillary leaves

The photoperiod change dictated the phyllochron and the time to axillary leaf appearance. The thermal time accumulation from sowing to the first axillary leaf appearance at Castle Hill, Lees Valley and Breach Oak did not comply with their phyllochron. For example, the phyllochron for plants sown in the Castle Hill (11th March 2011) and Lees Valley (29th March 2011) were similar (Figure 5.13) but plants in the Lees Valley had a lower thermal time requirement to produce their first axillary leaf than those grown in Castle Hill (Figure 5.16). In terms of the number of nodes to axillary leaf appearance (Appendix 8), Lees Valley plants had five nodes whereas Castle Hill plants had four nodes before initiation of axillary leaves in ‘Bolta’ balansa and ‘Prima’ gland clovers. Despite that, the Lees Valley plants still reached axillary leaf appearance earlier than those in the Castle Hill. Similarly, plants from Breach Oak that had a low phyllochron (Figure 5.13) had the highest thermal time accumulation to their first axillary leaf (Figure 5.16). This lack of compatibility could be due to the method in estimation of the time to first axillary leaf in those annual clovers (Section 5.2.4.5.4). It was unknown when exactly the first axillary leaf appeared in these annual clovers because the sites were visited less frequent due to remote locations. Hence, the time to axillary leaf was predicted based on the number of nodes to the first axillary leaf in Table 3.10.

The calibration experiments in Iversen 9 field, Lincoln University generated a more reliable model that was able to estimate time to axillary leaf production for these annual clover species grown at different locations. Data from other regions with different seasonal and environmental conditions would be required to validate the use of this model over a broader area.

5.4.5 Time to flowering

The flowering response to photoperiod at validation locations was accurately predicted (Figure 5.18). Regardless of location, plants that emerged in March – April flowered much later than those that emerged in January - February. In addition to the photoperiod effect, differences in temperatures among locations also accounted for the variation in thermal time accumulation throughout the vegetative period. For example, daily thermal time accumulation from Breach Oak was high due to warmer temperatures which meant fewer days required to flower (Table 5.17 and Table 5.18). Thermal time accumulation in Castle Hill was lower per day because temperatures were colder and therefore plants took more days but a consistent thermal time to flower.

In 'Mihi' Persian clover, flowering data from the additional sowing in Iversen 9 field and repeated sowings in the Lincoln University glasshouse gave a more complete picture of the flowering response (Figure 5.19d). The generated function could be be used to predict time to flowering for summer sown plants. This function needs to be validated at independent experiment sites. The flowering time for photoperiods >16.4 hours (Figure 5.19) remains to be quantified in CEC or field environments to determine the optimum photoperiod for this species.

The flowering model from the calibration experiments quantified the response of thermal time to flowering and photoperiod accurately across 10 sowing dates with seasonal variation in temperatures and daylength (Figure 4.4). The coefficient of determinant (R^2) of the model ranged from 43-97% for flowering response to increasing photoperiod, 93-100% and 82-99% for response to decreasing photoperiod into the autumn and winter (Table 4.5). This model, which was developed from field experiments, was also validated under field and glasshouse conditions across locations with variations in temperature and photoperiod. The low RMSD values (6.4-21.4%) highlight the accuracy of this model in the prediction of flowering time of these four annual clovers. It could be further improved if the date of seedling emergence was accurately determined at the remote locations (by counting seedlings more frequently during the first two weeks after sowing).

A similar model for flowering time of 'Bolta' balansa clover across six sowing dates in 2005-2006 was developed previously by Monks (2009) but using a base temperature (T_b) of 2.5 ^oC and a photoperiod response at emergence. However, quantification of flowering time using a

T_b of 2.5 ^oC was estimated from a limited range of temperatures and was higher than the physiological T_b for which development is zero (Bonhomme, 2000) (Section 3.5.4). Therefore, this model was validated in this study using a T_b of 0 ^oC and it conformed with the calibration model developed from experiments at Iversen 9 field, Lincoln University in 2010-2011. In contrast, the flowering model developed by Summerfield *et al*. (1991) has been widely used to predict time to flowering in annual crops (Craufurd *et al.*, 1999; Evans *et al.*, 1992). However, this model was developed in controlled environment conditions with constant photoperiod and is unable to account for the variation in flowering time in response to photoperiod direction when validated in field conditions.

5.5 Conclusions

1. Emergence for ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers in the CEC was consistent with the field derived model. In contrast, emergence for ‘Bolta’ balansa and ‘Mihi’ Persian clovers in the controlled environment was slower than in Iversen 9 field. The combined dataset gave a consistent estimation of seedling emergence for all species but needs to be validated with another independent dataset.

2. The appearance of first leaf in the CEC conformed with the calibration model. Reanalysis of combined field and CEC data generated a new model that provided an improved description of first leaf emergence for further validation.

3. The phyllochron validated at different locations and sowing dates conformed with the predicted pattern that leaf appearance responded to the length and direction of photoperiod change. Species sown in the CEC in a constant 16.0 hour photoperiod had a longer phyllochron and their leaf appearance tended to behave as though the photoperiod was decreasing. The calibration model was an accurate predictor of the phyllochron of the annual clovers grown at different locations.

4. The observed time to axillary leaf appearance followed the pattern in the calibration model. The model was reliable and able to estimate time to axillary leaf production for annual clover species grown at any given date and location.

5. In all species, the thermal time requirement to flowering for plants sown in all locations was consistent with the derived model. For ‘Mihi’ Persian clover, a predicted model for plants sown in the summer into a decreasing photoperiod was generated. The flowering gap beyond the longest field photoperiod of 16.4 hours requires quantification in a different environment to determine the optimum photoperiod for flowering in this species.

6. The CEC is a suitable environment to quantify time of emergence and first leaf because it can extend the range of temperatures at the supra-optimal range and minimize undesirable variations in the field conditions. However, the CEC was less practical to quantify phyllochron and time to flowering because plants do not grow under natural conditions due to the lack of photoperiod change and direction.

6 Dry matter production, light interception and radiation use efficiency

6.1 Introduction

Under optimum growth conditions, crop yield is dependent on the amount of photosynthetically active radiation (PAR) intercepted by the green leaf canopy (Monteith, 1972). Specifically, the accumulation of crop dry matter production is proportional to the accumulation of intercepted PAR (Kiniry *et al.*, 1989) and therefore, the slope of this fitted relationship represents the radiation use efficiency (Sinclair and Muchow, 1999) (Section 2.10.1).

In this chapter, the growth of these four annual clovers in relation to canopy expansion, PAR interception and dry matter production is described. The objectives were (1) to quantify dry matter production from four annual clovers sown on four dates, (2) to quantify crop radiation use efficiency from the conversion of PAR captured into dry matter and (3) to examine the regeneration of crop biomass after the first year.

6.2 Materials and methods

The experimental area was located in Block 9 of Iversen field (Iversen 9) at Lincoln University, Canterbury, New Zealand (43^o 38'S, 172^o 28'E, 11 m a.s.l.). Information on the site description and meteorological conditions were provided in Chapter 3 (Section 3.2.2).

6.2.1 Experimental design

This second field experiment was established as a split-plot within a randomised complete block design. The main-plots were four sowing dates (Table 6.1) replicated four times and the subplots were the four annual clover species ('Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian). Each subplot measured 4.2 x 10 m, giving an area of 42 m^2.

6.2.2 Sowing method

Prior to sowing, the area was cultivated using conventional methods to produce a firm and fine textured seedbed. Bare seeds of the four clover species were sown as pure stands at 6 kg/ha for 'Cefalu' arrowleaf, 4 kg/ha for 'Bolta' balansa and 'Prima' gland and 5 kg/ha for 'Mihi' Persian based on germination test. All seeds were sown with Group C inoculants (sourced: ALOSCA Tech. Pty. Ltd., Australia). The plots were drilled with an Øyjoord cone seeder with chain harrows following, at 150 mm row spacing and at a target depth of 10 mm.

6.2.3 Management

Plots sown on 26 February 2010 (SD1) were sprayed on 3rd June 2010 with a mixture of Preside (a.i. 800 g/kg flumetsulam) at 65 g/ha and 'Hasten' wetting agent at 2 L/ha to control broad leaf weeds, particularly fathen (*Chenopodium album*). On 17th June 2010, Centurion Plus (a.i. 125 g/L clethodim) was applied to plots sown on 26th February 2010 and 24th March 2010 (SD2) at 1 L/ha to control grass weeds. No irrigation was applied and plots were not grazed throughout the experimental period to allow maximum seed set. In the following year (2011), when all the seed pods had shattered, the dead herbage was removed on 29 April 2011, using a rotary mower to facilitate seedling establishment. All plots were sprayed on 1st August 2011 with Gallant Ultra (a.i. 520 g/L haloxyfop-P) at 250 mL/ha to control grass weeds. The experimental area remained ungrazed and clover seedlings were left to grow to flowering for the second season.

6.2.4 Measurements

6.2.4.1 Thermal time

Measurement of temperatures and thermal time calculation were described in Section 3.2.2.5.1. For each sowing date, air temperatures were monitored from seedling emergence to full flower (Figure 6.1).

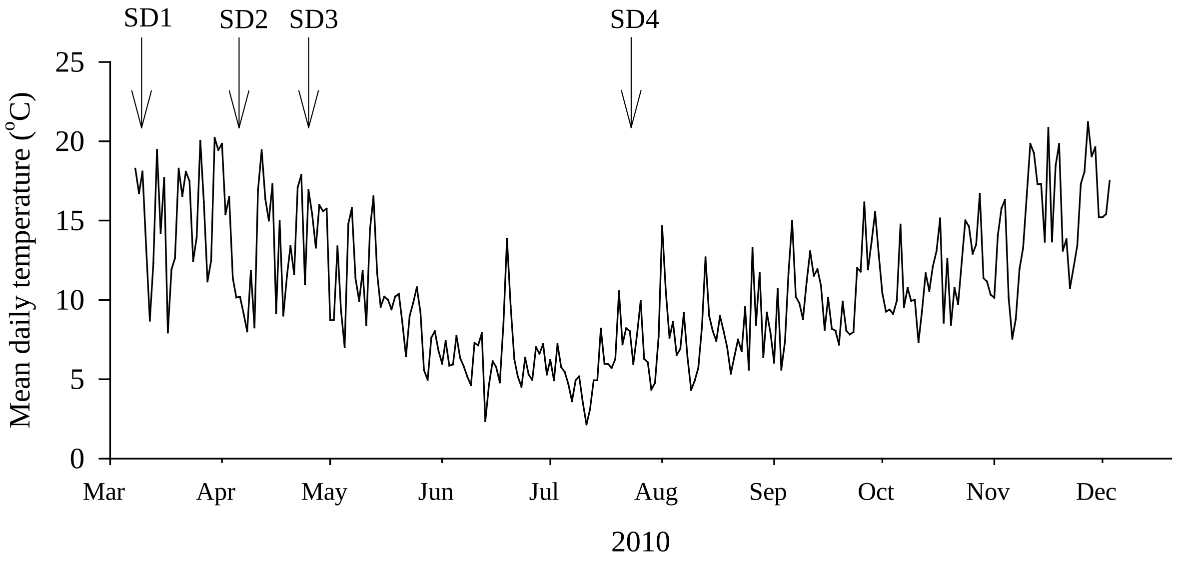


Figure 6.1 Mean daily air temperatures from emergence to full flower for annual clovers sown on four dates (SD) at Iversen 9 field, Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10, SD2: 24 Mar 10, SD3: 19 Apr 10, SD4: 19 Apr 10.

6.2.4.2 Seedling growth and development

After sowing, two adjacent one meter sections of non-border rows were marked on each subplot. The number of seedlings which emerged within the row was counted every alternate day until seedlings ceased to emerge. Time of emergence was determined when 50% of seedlings within the row had emerged. At the first trifoliate leaf stage, the number of seedlings were counted again to quantify plant population.

When the seedlings had 3-4 leaves, 10 adjacent seedlings in a drill row were destructively harvested at bi-weekly intervals until ~1200 °C d after emergence. The harvest dates for each sowing are shown in Table 6.1.

Table 6.1 Harvest dates for seedlings sown on four dates in 2010 at Lincoln University, Canterbury, New Zealand.

Sowing date (2010)	Harvest dates (2010)
26 Feb (SD1)	8 Apr, 23 Apr, 11 May, 14 Jun, 2 Aug
24 Mar (SD2)	7 Jul, 17 Aug, 16 Sep
19 April (SD3)	31 Jul, 17 Aug, 16 Sep, 29 Sep
8 Jul (SD4)	16 Sep, 29 Sep, 20 Oct, 3 Nov, 23 Nov

Individual seedling height, leaf number and dry weight were measured. Seedling height was measured with a ruler from the base to the highest tip of the plant. The internode length was calculated from a fitted regression between the seedling height and number of leaves on the main stem. The slope of the fitted relationship represents the internode length. The seedlings were then dried in a force-draught oven at 60°C for at least 48 hours before dry weight determination.

6.2.4.3 Dry matter production

Dry matter (DM) was measured bi-monthly during winter and bi-weekly during spring and summer. Measurements started when the crop height reached ~70 mm and ended when crops had surpassed full flower (stage 5 (Table 4.1 to Table 4.4). Two measurements were taken from each subplot by using a 0.2 m^2 quadrat, cut to 30 mm above ground with hand shears. For clovers that regenerated in 2011 (Section 7.2.2), the DM was measured once, at the full flower stage. The herbage samples were mixed together and a sub-sample (about 50 g of fresh weight) was sorted into clover and weeds. The herbage was then dried in a force-draught oven at 60 – 70°C to constant weight and the dry weight for each herbage component measured using an electronic scale.

6.2.4.4 Light interception

Canopy light interception was measured at 14-21 day intervals using a SunScan Canopy Analysis System (Delta-T Devices, Cambridge, England), starting from when the canopy reached a height of ~ 70 mm. The SunScan uses a set of algorithms to compute leaf area index (L) from the proportion of incident and transmitted photosynthetically active radiation (PAR) within the crop canopy (Webb *et al.*, 2008). The leaf area index is not reported because no independent data set was measured to validate the Sunscan's calculation and the main measurement required was light interception.

6.2.5 Calculations and data analysis

6.2.5.1 Dry matter accumulation

Dry matter accumulation in relation to thermal time was described using a logistic growth function described in Equation 6.1 (Loss *et al.*, 1989).

Equation 6.1 $$Y = A + \frac{C}{(1 + e^{(-B(X-M))}}$$

Where:

A = initial biomass at time 0.
C = maximum biomass
B = slope/ rate of biomass increment
M = time at maximum growth rate
X = thermal time

The lag phase, defined as the duration to accumulate 5% of total dry matter was calculated by re-arranging Equation 6.1 where thermal time (X) was made as the dependent variable, when y = 5% of total dry matter, as shown in Equation 6.2.

Equation 6.2 $$X = \left[\frac{\ln\left(\frac{C}{Y}-1\right)}{-B}\right] + M$$

Similarly, the duration to reach 95% of total dry matter was calculated using the same equation by modifying y = 95% of total dry matter.

6.2.5.2 Intercepted PAR

The fraction of intercepted PAR (τ) was calculated based on procedures outlined by Gallagher and Biscoe (1978) and shown by Equation 6.3:

Equation 6.3 $$\tau = 1.0 - \beta$$

where, β is the fraction of transmitted PAR.

Incident PAR (φ, MJ m^{-2} d^{-1}) was assumed to be close to 50% of total solar radiation (Monteith, 1972). Daily τ was estimated from interpolation of the measurements of τ against time (in days). Daily intercepted PAR (PAR_i) and total PAR_i were calculated from Equation 6.4 based on O'Connel *et al*., (2004) as:

Equation 6.4 $$\text{Daily } PAR_i = \text{daily } \tau * \text{daily } \varphi$$

Total PAR_i was determined as the sum of daily PAR_i from emergence to the final date of crop harvest.

6.2.5.3 Canopy expansion

The canopy expansion was described by the increment amount of intercepted PAR in relation to time. To determine the duration (^{o}C d) to reach 95% of total intercepted PAR, a logistic function (Equation 6.1) was fitted from the relationship between fraction of intercepted PAR (τ) and thermal time accumulation. The duration (^{o}C d) to reach 95% of total intercepted PAR was calculated from Equation 6.2, where $y = 0.95$.

6.2.5.4 Radiation use efficiency (RUE)

Absorbed PAR was assumed to be equal to 0.85 of total PAR_i (Sinclair and Muchow, 1999). Radiation use efficiency (RUE) was calculated as the slope of a fitted linear relationship between DM accumulation and total absorbed PAR (Sinclair and Muchow, 1999). For this calculation, the DM accumulation includes weeds because the measurement of light interception was conducted in the field plots where weeds were present. The regression was constrained to intercept the axis at the origin (0,0) on the basis that at 0 MJ/m^2 of absorbed PAR, the crop has a biomass of 0 g DM/m^2 (Fletcher *et al*., 2008).

6.2.5.5 Root to shoot ratio

The root to shoot ratio was measured from plants grown in pots in a controlled environment chamber (Section 5.2.1). This measurement used plants from pots instead of the field because of the feasibility of extracting whole root systems rather than losing any root components from deep in the soil. The aim was to quantify the changes in seedling carbon partitioning in response to the environment experienced by the plants during vegetative growth. The experimental design for this measurement was described in Chapter 5. Briefly, four species of annual clovers (‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’Persian) with four replicates were grown in 4.5 L pots in a completely randomized design. Each experiment was conducted in a controlled environment chamber at one of five different temperature regimes: 9/6, 15/6, 20/10, 25/15 and 30/20 ^{o}C (Table 5.1). The plants were grown under an 8/8 h temperature and photoperiod regime with 4 h transitions between day and night. Light reaching the plant canopies was 448 ± 9 µmoles/m^{2}/s photosynthetic photon flux density (PPFD) (Section 5.2.1). The plants in each pot were destructively harvested at the end of the experiment, when they had produced 6-8 leaves on the main stem. The plants were then washed and separated into shoots and roots. These shoots and roots were dried in a forced-draught oven at ~60 ^{o}C to a constant weight. The root to shoot ratio was calculated from Equation 6.5:

Equation 6.5

$$\frac{\text{Root weight (g)}}{\text{Shoot weight (g)}}$$

6.3 Results

6.3.1 Total dry matter accumulation

Maximum total accumulated dry matter was 17.5 (±2.07) t/ha in SD1 (26 Feb 10) for 'Bolta balansa, 12.5 (±1.24) t/ha in SD1 for 'Mihi' Persian, 11.0 (±1.48) t/ha in SD3 (19 April 10) for 'Prima' gland and 9.4 (±0.75) t/ha in SD1 for 'Cefalu' arrowleaf (Figure 6.2). The regenerated dry matter production for year two at full flower averaged 11.6 (±0.73) t/ha for 'Bolta' balansa, 8.3 (±1.11) t/ha for 'Mihi' Persian and 0.5 (±0.17) t/ha for 'Cefalu' arrowleaf clover across all sowing dates. For 'Prima' gland clover, the regenerated dry matter production ranged from 1.6 (±0.18) t/ha in SD1 to 2.9 (±0.24) t/ha in SD2 (24 Mar 10) (Figure 6.2c). The percentage of weeds in each plot at full flower is shown in Table 6.2.

Table 6.2 Percentage of weeds (%) at full flower stage of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Percentage of weeds (%) when annual clovers at full flower			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26 Feb 10	2	3	7	1
24 Mar 10	35	10	22	10
19 Apr 10	28	6	7	8
8 Jul 10	44	37	32	20

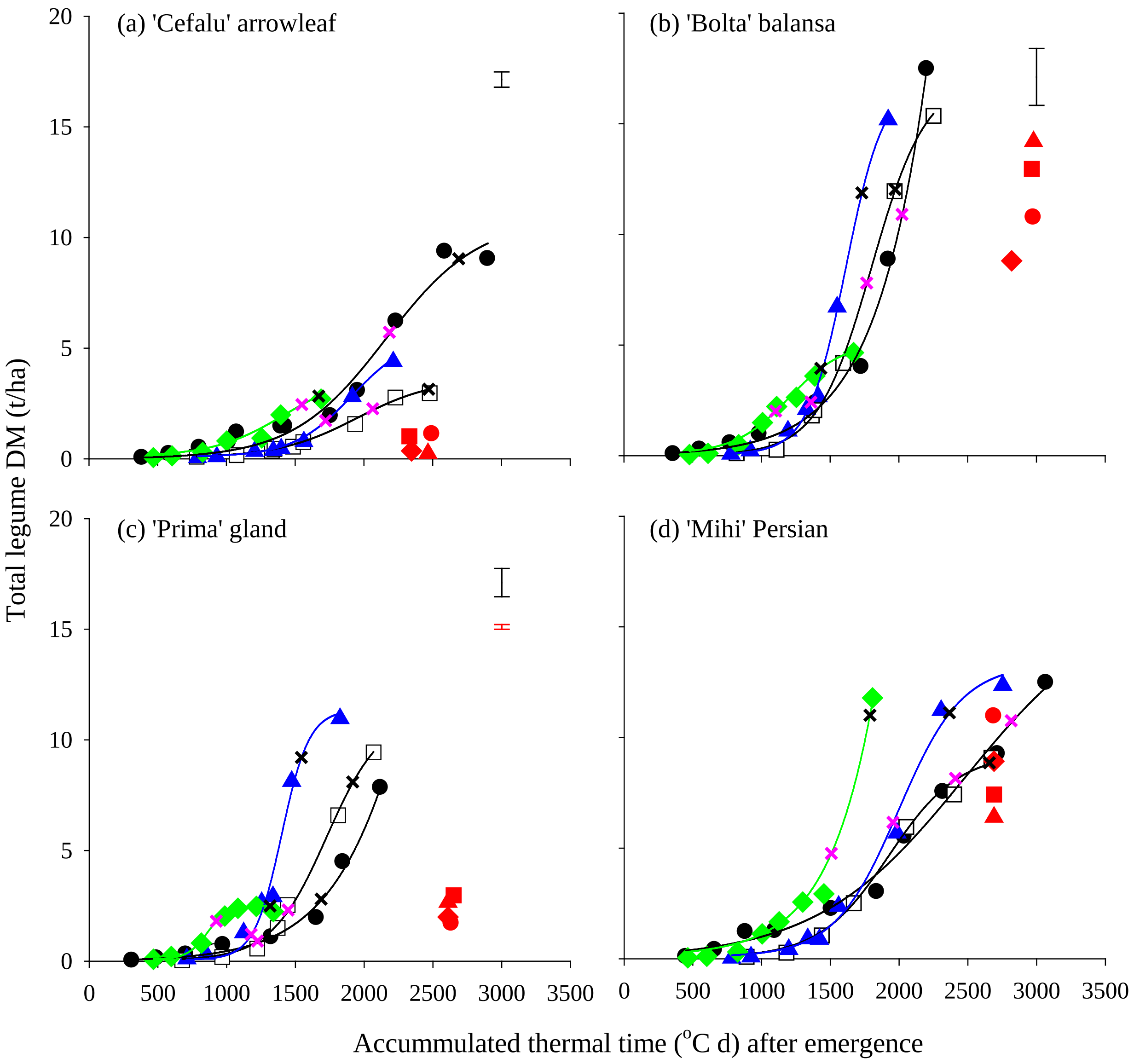


Figure 6.2 Accumulated legume dry matter (t/ha) of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●,🔴), SD2: 24 Mar 10 (□,🟥), SD3: 19 Apr 10 (▲,🔺), SD4: 8 Jul 10 (◆,♦). Full flower (✕), Physiological maturity (✕). Red symbols are the dry matter at full flower from the regenerated annual clovers in year two (2011). Bars represent one standard error of the mean where sowing dates were different ($P<0.05$); black bars represent sowing dates in 2010 and red bar represents regenerated clover in year two (2011). Note: Thermal time calculation used air temperature (T_b = 0 °C).

6.3.1.1 Dry matter at full flower

The dry matter accumulation at full flower was measured in year 1 (2010) to allow direct comparison with results in year 2 (2011). The yield was affected by a sowing date x species interaction (Table 6.3). The highest yield at full flower was from crops sown on 26 Feb 10 (SD1), except for 'Prima' gland clover which had the highest yield from SD2 (24 Mar 10). The dry matter ranged from 1.6 to 5.6 t/ha for 'Cefalu' arrowleaf, 1.9 to 10.8 t/ha for 'Bolta' balansa, 0.8 to 2.2 t/ha for 'Prima' gland and 4.6 to 10.6 t/ha for 'Mihi' Persian clovers.

Table 6.3 Dry matter at full flower for 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Dry matter accumulation at full flower (t/ha)			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26 Feb	5.6	10.8	0.8	10.6
24 Mar	2.1	7.7	2.2	8.0
19 Apr	1.6	2.3	1.1	6.0
8 Jul	2.3	1.9	1.7	4.6
Mean	2.9	5.7	1.5	7.3
	SD	Species	SD*Species	
P- value	<0.001	<0.001	<0.001	
V.r.	34.76	69.75	10.23	
S.E.M.	0.35	0.32	0.65	
Except when comparing means at the same SD			0.63	
L.S.D. (5%)	1.11	0.90	1.85	
Except when comparing means at the same SD			1.81	

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

6.3.1.2 Duration of lag phase in dry matter accumulation

The duration of the lag phase or time (^{o}C d) to accumulate 5% of total crop dry matter was affected (P<0.001) by a sowing date x species interaction (Table 6.4). In 'Cefalu' arrowleaf, 'Bolta' balansa and 'Prima' gland clovers, crops sown on 26 Feb 10 (SD1) had the longest duration of lag phase where as SD4 crops accumulated 5% of their total dry matter within the shortest time. For 'Mihi' Persian clover, the duration of it's lag phase averaged 1195 ^{o}C d across all four sowing dates.

Table 6.4 Duration of lag phase (^{o}C d) of dry matter accumulation for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Duration of lag phase (^{o}C d) of dry matter accumulation			
(SD)	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	1433	1382	1267	981
24 Mar	1155	1323	1168	1213
19 Apr	1356	1284	1141	1326
8 Jul	822	758	580	1114

	SD	Species	SD*Species
P- value	<0.001	<0.041	<0.001
V.r.	45.8	3.1	4.2
S.E.M.	32.3	40.9	77.8
Except when comparing means at the same SD			81.7
L.S.D. (5%)	103.4	117.2	221.6
Except when comparing means at the same SD			234.4

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

6.3.1.3 Duration to 95% of dry matter accumulation

The duration to accumulate 95% total yield differed with sowing date and species (P<0.001). In all species, the duration was shortened with successive sowing dates from 3221 ^{o}C d in SD1 (26 Feb) to 1551 ^{o}C d in SD4 (8 Jul) for ‘Cefalu’ arrowleaf clover (Table 6.5). In comparison among species, ‘Prima’ gland clover had the shortest duration in accumulation of it’s total biomass (1338- 2503 ^{o}C d). In contrast, ‘Mihi’ Persian clover had the longest period (2011 – 3894 ^{o}C d) of dry matter accumulation.

Table 6.5 Duration (^{o}C d) from 0 to 95% of dry matter accumulation for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date (SD)	Duration (^{o}C d) to 95% of dry matter accumulation ‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	3221	2728	2503	3894
24 Mar	2884	2155	2282	2744
19 Apr	2402	1914	1711	2750
8 Jul	1551	1572	1338	2011
	SD	Species	SD*Species	
P- value	<0.001	<0.001	0.188	
V.r.	41.9	20.7	1.5	
S.E.M.	94.8	89.4	181.5	
Except when comparing means at the same SD			178.7	
L.S.D. (5%)	303.3	256.3	-	
Except when comparing means at the same SD			-	

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

6.3.2 Intercepted PAR and canopy expansion

The relationship between total PAR$_i$ and thermal time accumulation was described by a bi-linear regression (Figure 6.3). In all species, crops sown on 8 Jul 10 (SD4) had the shortest duration of lag phase (Table 6.6). The end of lag phase marks the beginning of rapid canopy expansion. In contrast, SD1 (26 Feb 10) crops took the longest time to commence this phase. The linear rate of canopy expansion, represented by the rate of PAR$_i$ per heat unit was between 0.5-0.8 MJ m^{-2} ^{o}C d^{-1} for ‘Cefalu’ arrowleaf, 0.7-0.9 MJ m^{-2} ^{o}C d^{-1} for both ‘Bolta’ balansa and ‘Prima’ gland clovers and 0.6-0.8 MJ m^{-2} ^{o}C d^{-1} for ‘Mihi’ Persian clover.

In ‘Cefalu’ arrowleaf clover, only crops sown on 26 Feb 10 (SD1) managed to intercept 95% PAR after 2224 ^{o}C d whereas those sown on 24 Mar (SD2), 19 Apr (SD3) and 8 Jul 10 (SD4) did not reach 95% PAR$_i$ before maturity (Figure 6.4a). For the other three species, the duration from seedling emergence to reach 95% of PAR$_i$ by the crop canopy ranged from 1553 (SD2) to 1728 ^{o}C d (SD1) in ‘Bolta’ balansa, 1484 (SD3) to 1979 ^{o}C d (SD1) in ‘Prima’ gland and ~2007 ^{o}C d (SD1-SD3) in ‘Mihi’ Persian clover. In all species, SD4 crops matured before their canopy was completely closed (Figure 6.4).

Table 6.6 Duration of lag phase (°C d) and rate of linear canopy expansion (MJ m^{-2} $^{o}Cd^{-1}$) described by a fitted regression between total PAR_i (MJ m^{-2}) and thermal time (°C d) of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover sown on four dates (SD) in 2010 at Lincoln University, New Zealand.

Species	SD (2010)	Duration of lag phase[1] ± S.E. (°C d)	Rate of canopy expansion ± S.E. (MJ m^{-2} ^{o}C d^{-1})	R^2 (%)
'Cefalu' arrowleaf	26 Feb	1683 ± 27.7	0.82 ± 0.026	99.6
	24 Mar	1697 ± 39.0	0.66 ± 0.031	99.7
	19 Apr	1472 ± 43.3	0.62 ± 0.030	99.5
	8 Jul	941 ± 60.6	0.46 ± 0.016	99.7
'Bolta' balansa	26 Feb	1577 ± 35.9	0.82 ± 0.049	99.4
	24 Mar	1421 ± 16.7	0.91 ± 0.018	99.9
	19 Apr	1259 ± 22.7	0.88 ± 0.020	99.9
	8 Jul	878 ± 34.5	0.72 ± 0.014	99.9
'Prima' gland	26 Feb	1565 ± 22.6	0.78 ± 0.035	99.6
	24 Mar	1311 ± 17.4	0.91 ± 0.019	99.9
	19 Apr	1184 ± 22.8	0.91 ± 0.023	99.8
	8 Jul	866 ± 34.5	0.72 ± 0.024	99.9
'Mihi' Persian	26 Feb	1703 ± 29.3	0.81 ± 0.024	99.7
	24 Mar	1490 ± 23.0	0.75 ± 0.014	99.9
	19 Apr	1271 ± 29.3	0.69 ± 0.008	99.9
	8 Jul	1199 ± 67.5	0.59 ± 0.031	99.7

SD, sowing date. S.E., standard error. R^2, coefficient of determination. [1]The end of lag phase marks the beginning of linear canopy expansion.

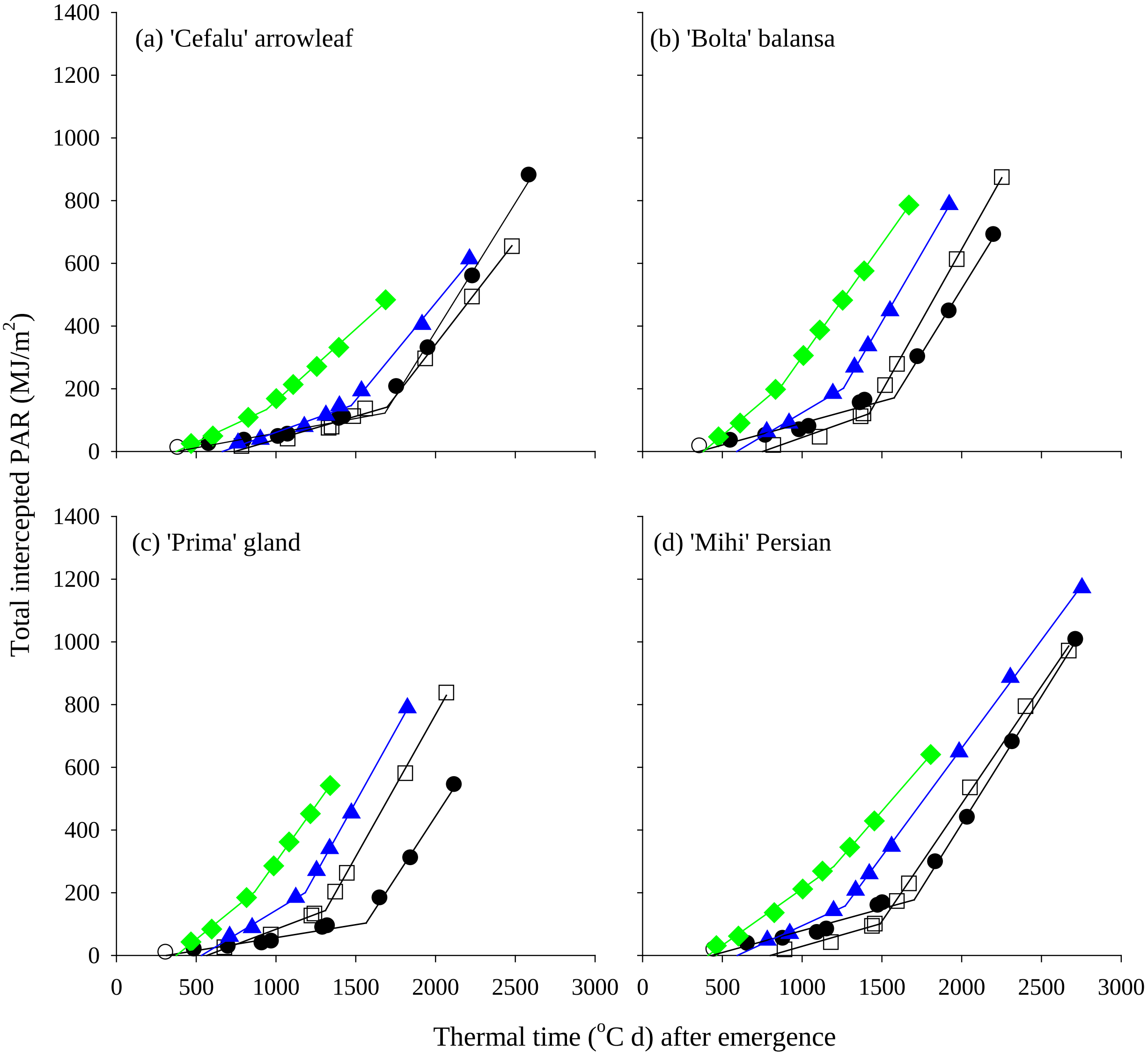


Figure 6.3 Total intercepted photosynthetically active radiation (PAR) versus accumulated thermal time after emergence for (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (☐), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). For each fitted line, the break point indicates beginning of canopy expansion. Note: Thermal time calculation used air temperature (T_b = 0 °C).

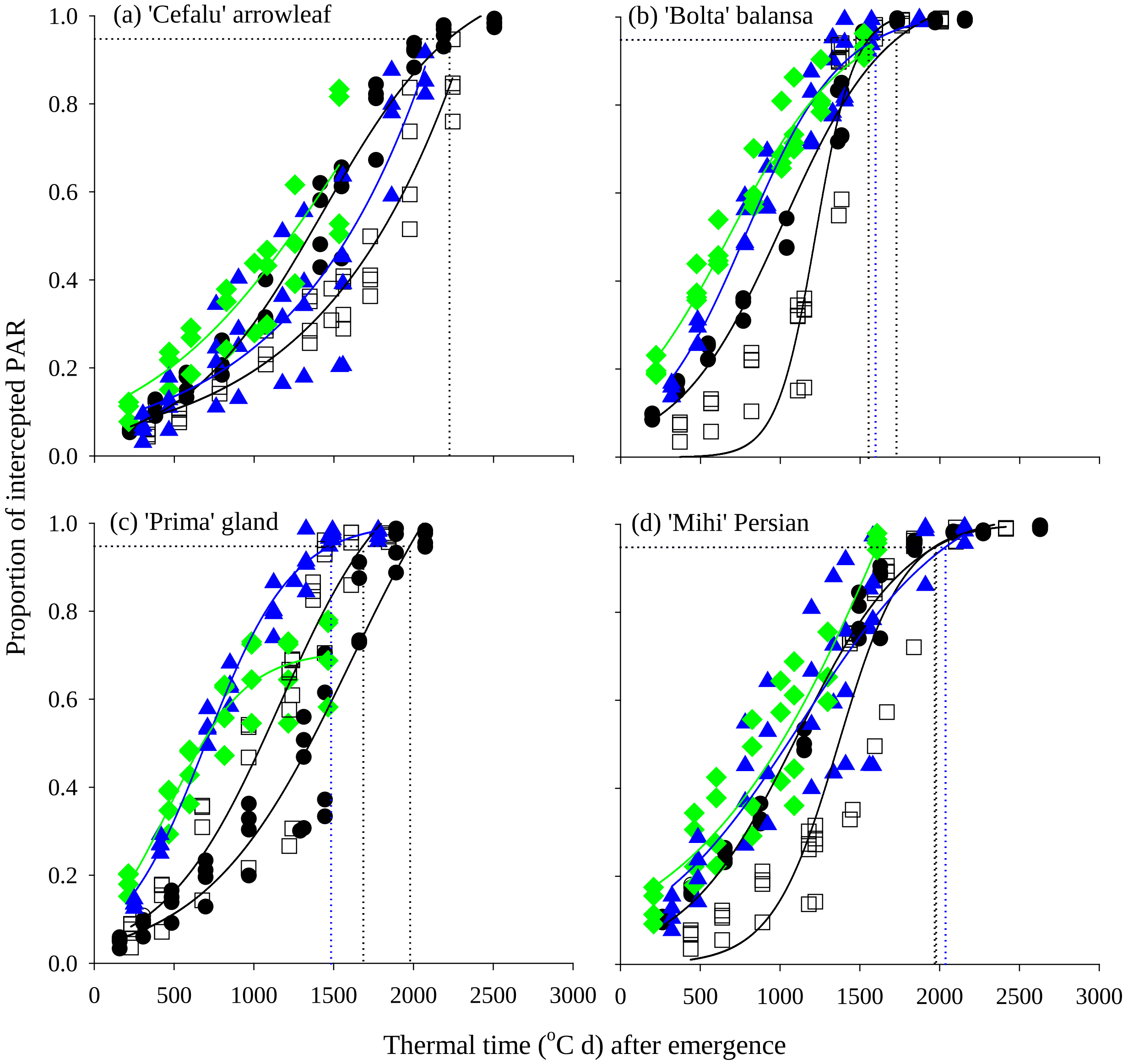


Figure 6.4 Proportion of intercepted photosynthetically active radiation (PAR) versus accumulated thermal time for (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). The solid line represents the fitted relationship and the dotted 95% PAR intercepted and thermal time, respectedly. Thermal time calculation used air temperature (T_b = 0 °C).

6.3.2.1 Total intercepted PAR (PAR$_i$)

There was an interaction (P<0.001) between the sowing date and species in the total intercepted PAR. In 'Cefalu' arrowleaf clover, total PAR$_i$ decreased with successive sowing dates from 880 MJ/m^2 in SD1 (26 Feb 10) to 484 MJ/m^2 in SD4 (8 Jul 10) (Table 6.7). 'Bolta' balansa clover sown on 24 Mar 10 (SD2) intercepted the highest PAR of 875 MJ/m^2. 'Prima' gland clover sown on 24 Mar 10 (SD2) and 19 Apr 10 (SD3) both intercepted ~814 MJ/m^2 PAR. 'Mihi' Persian clover had the lowest PAR intercepted (641 MJ/m^2) by crops sown on 8 Jul 10 (SD4).

Table 6.7 Total intercepted PAR (PAR$_i$) by 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Total PAR$_i$ (MJ/m^2)			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26 Feb	880	690	543	1006
24 Mar	655	875	838	972
19 Apr	614	787	789	1172
8 Jul	484	786	542	641
	SD	Species	SD*Species	
P- value	<0.001	<0.001	<0.001	
V.r.	14.6	62.3	18.3	
S.E.M.	27.8	16.8	40.3	
Except when comparing means at the same SD			33.6	
L.S.D. (5%)	89	48.2	116.2	
Except when comparing means at the same SD			96.4	

V.r. Variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

6.3.3 Seedling establishment

To explain the variation in dry matter production and canopy expansion among the sowing dates and species, plant population, seedling height, internode length and leaf appearance were measured.

6.3.3.1 Plant population

The number of plants/m^2 was affected by the sowing date x species interaction (P<0.004) with the lowest plant population from seeds sown on 24 Mar 10 (SD2) (Table 6.8). In most cases, emergence from 24 Mar (SD2) appeared to be affected by the low moisture for a period of more than a week following sowing which hampered seed germination. The plant population constituted about 17 - 32% (‘Cefalu’ arrowleaf), 30 - 60% (‘Bolta’ balansa), 22 - 51% (‘Prima’ gland) and 22 - 45% (‘Mihi’ Persian) seedling emergence from the number of seeds sown.

Table 6.8 Plant population/m^2 and their percentage (%, in brackets) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Plant population/m^2 and their percentage (%, in brackets)			
(SD)	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	236 (31.9)	283 (59.9)	375 (48.3)	133 (39.6)
24 Mar	125 (16.9)	144 (30.5)	173 (22.2)	75 (22.4)
19 Apr	178 (24.0)	235 (49.7)	393 (50.6)	117 (34.9)
8 Jul	172 (23.2)	142 (30.0)	317 (40.8)	149 (44.6)
	SD	Species	SD*Species	
P- value	<0.002	<0.001	<0.004	
V.r.	12.4	48.5	3.4	
S.E.M.	15.7	11.8	25.8	
Except when comparing means at the same SD			23.6	
L.S.D. (5%)	50.2	33.8	73.7	
Except when comparing means at the same SD			67.6	

V.r. Variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences. Date of measurement was on 11 April 2010 for SD1 (26 Feb), 29 May 2010 for SD2 (24 Mar), 22 June 2010 for SD3 (19 Apr) and 11 September 2010 for SD4 (8 Jul).

6.3.3.2 Seedling height

At 1200 ^{o}C d after emergence, SD4 (8 July 10) crops that established through the spring were the tallest (Figure 6.5 and Figure 6.6). SD1 (26 Feb 10) seedlings had faster growth during autumn but grew slowly in winter (Figure 6.5). In contrast, SD2 (24 Mar 10) and SD3 (19 April 10) seedlings had slow growth during early establishment in winter but accelerated exponentially in the spring.

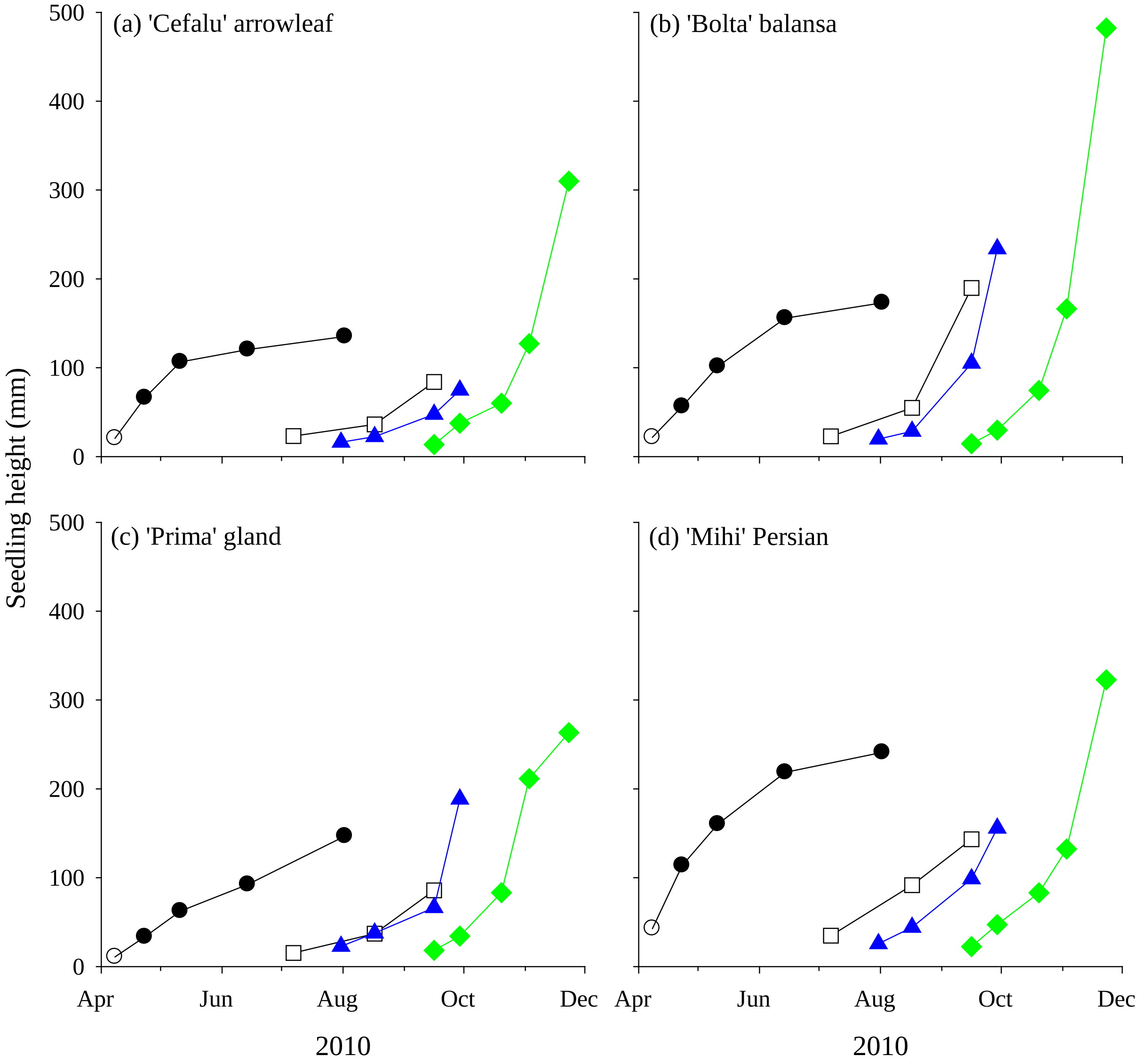


Figure 6.5 Seedling height over time of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (☐), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆).

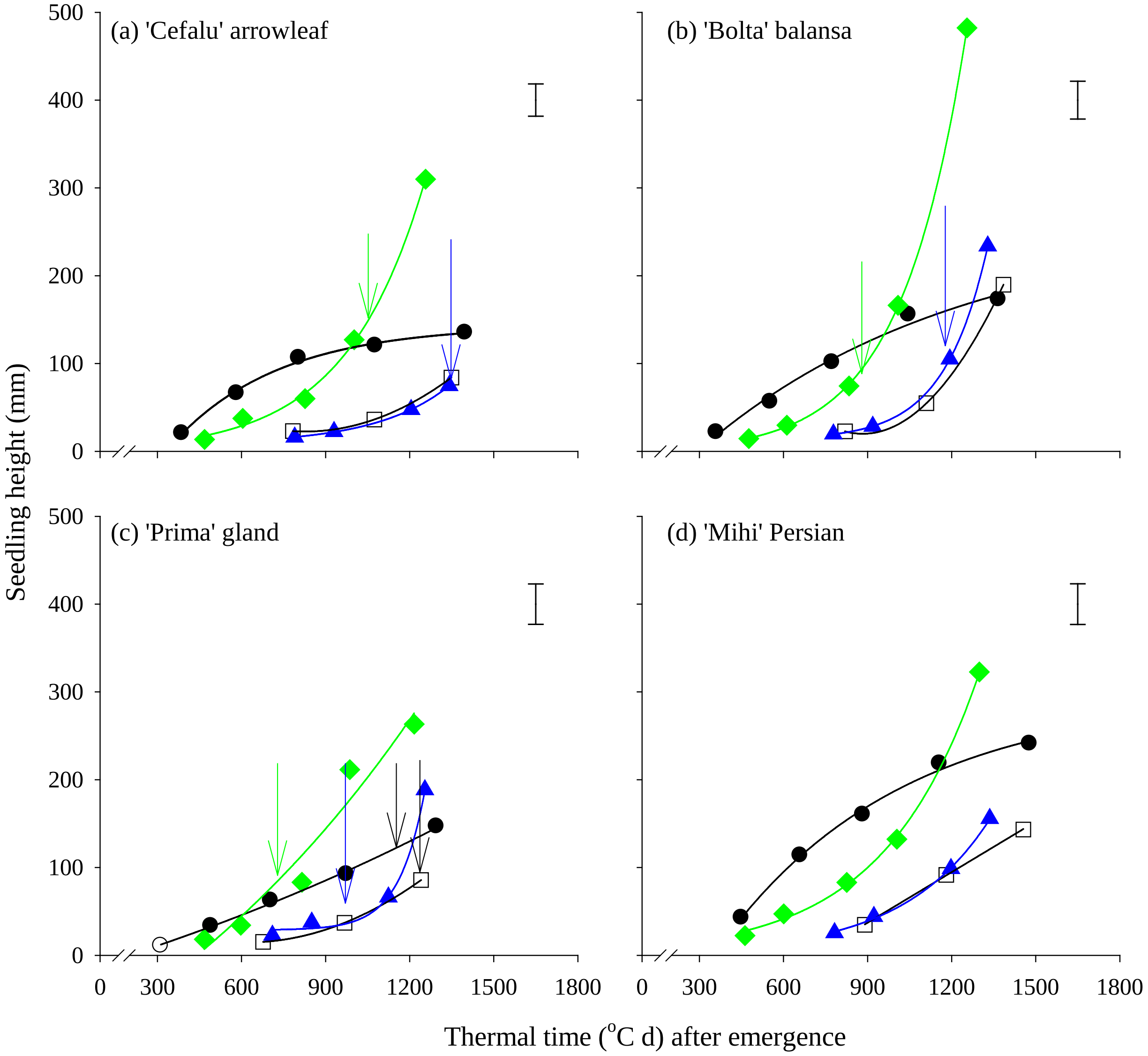


Figure 6.6 Seedling height versus thermal time after emergence of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). Arrows indicate time of flowering (first bud visible). Error bars represent the maximum standard error for the final seedling height. Thermal time calculation used air temperature (T_b = 0 °C).

6.3.3.3 Internode length

In all species, the internode length differed with sowing dates (Table 6.9). This was mainly due to the differences in daily mean air temperature during the seedling growth. For example, the internode elongated with increased temperature from 11.6 mm at 8.3 °C (SD1) to 36.7 mm at 12.3 °C (SD4) in'Bolta' balansa clover.

Table 6.9 Internode length (mm) from fitted regression between plant height and leaf appearance on the main stem of Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on four dates (SD) in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Species	Sowing date (SD)	Internode length ± S.E. (mm)	R^2 (%)	Mean Temperature (°C)	[1]DT/[2]NT (°C)
'Cefalu' arrowleaf	26 Feb	14.9 ± 1.7	95	8.3	12.6/4.0
	24 Mar	13.3 ± 2.29	92	8.8	13.1/4.5
	19 Apr	14.9 ± 2.95	86	9.6	14.2/4.9
	8 Jul	23.1 ± 5.62	80	12.3	17.8/6.7
'Bolta' balansa	26 Feb	11.6 ± 3.19	75	8.3	12.6/4.0
	24 Mar	23.3 ± 6.85	84	8.8	13.1/4.5
	19 Apr	30.2 ± 7.21	81	9.6	14.2/4.9
	8 Jul	36.7 ± 9.12	79	12.3	17.8/6.7
'Prima' gland	26 Feb	10.6 ± 0.61	99	8.3	12.6/4.0
	24 Mar	18.1 ± 7.93	58	8.8	13.1/4.5
	19 Apr	18.5 ± 7.29	64	9.6	14.2/4.9
	8 Jul	22.8 ± 3.15	93	12.3	17.8/6.7
'Mihi' Persian	26 Feb	16.9 ± 2.41	92	8.3	12.6/4.0
	24 Mar	21.3 ± 5.96	80	8.8	13.1/4.5
	19 Apr	20.2 ± 2.44	94	9.6	14.2/4.9
	8 Jul	24.1 ± 4.83	86	12.3	17.8/6.7

S.E., standard error. R^2, coefficient of determination. [1]DT, day temperature. [2]NT, night temperature.

6.3.3.4 Seedling leaf appearance

Following sowing, the initiation of the first axillary leaf began between 696 (SD4) and 872 (SD2) ^{o}C d in ‘Cefalu’ arrowleaf, 455 (SD4) and 828 (SD2) ^{o}C d in ‘Bolta’ balansa, 453 (SD4) and 705 (SD2) ^{o}C d in ‘Prima’ gland and 561 (SD4) and 794 (SD2) ^{o}C d in ‘Mihi’ Persian clover (Figure 6.7). The appearance of axillary leaves began after five leaves had been produced on the main stem of ‘Cefalu’ arrowleaf clover and after the initiation of four leaves in ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers. In all species, the phyllochron decreased with each successive sowing date with the longest phyllochron observed for crops sown on 26 Feb 10 (SD1) and the shortest in SD4 (8 Jul 10) crops (Table 6.10). ‘Prima’ gland had the fastest leaf production (up to 84 leaves) where as ‘Cefalu’ arrowleaf clover had the slowest leaf production (up to 30 leaves) throughout the observation period (Figure 6.7).

Table 6.10 Phyllochron (^{o}C d/leaf) from fitted regression between leaf appearance on the main stem and thermal time (^{o}C d) of Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates (SD) in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Species	Sowing date (SD)	Phyllochron ± S.E. (^{o}C d/leaf)	R^2 (%)
‘Cefalu’ arrowleaf	26 Feb	118 ± 9.1	96.0
	24 Mar	85 ± 2.6	99.4
	19 Apr	81 ± 2.9	99.1
	8 Jul	67 ± 1.5	99.6
‘Bolta’ balansa	26 Feb	78 ± 5.5	96.7
	24 Mar	72 ± 3.0	98.9
	19 Apr	66 ± 3.0	98.6
	8 Jul	62 ± 1.6	99.6
‘Prima’ gland	26 Feb	75 ± 3.0	98.9
	24 Mar	74 ± 1.1	99.9
	19 Apr	68 ± 1.7	99.5
	8 Jul	63 ± 1.0	99.8
‘Mihi’ Persian	26 Feb	89 ± 1.1	99.9
	24 Mar	76 ± 2.5	99.3
	19 Apr	74 ± 1.0	99.9
	8 Jul	70 ± 0.7	99.9

S.E., standard error. R^2, coefficient of determination. Thermal time used air temperature (T_b = 0 ^{o}C) and calculated starting from first leaf appearance.

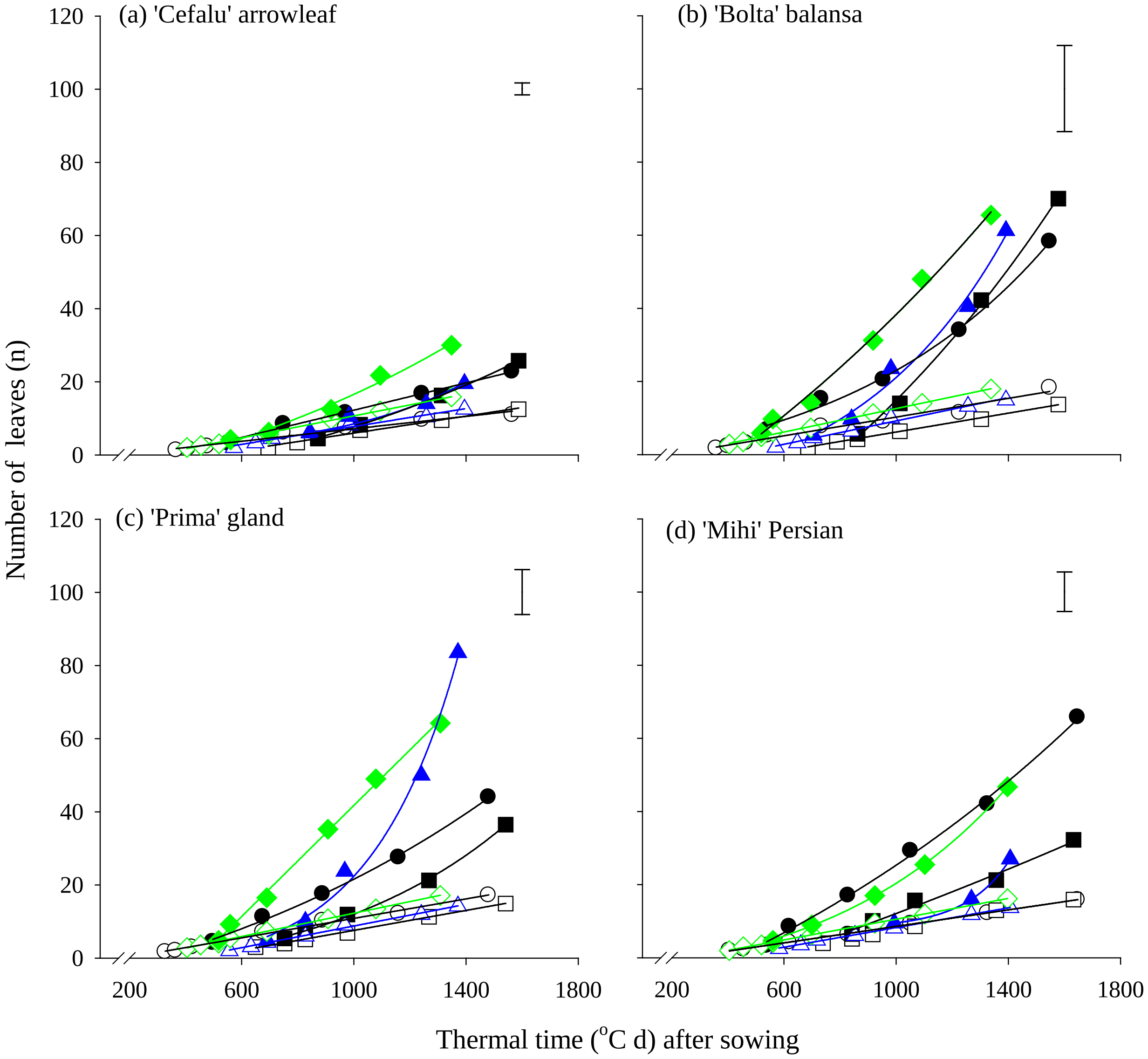


Figure 6.7 Number of total (closed symbols) and main stem (open symbols) leaves versus thermal time after sowing of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). Error bars represent the maximum standard error for the final total leaf number. Note: Thermal time calculation used air temperature (T_b = 0 °C).

6.3.3.5 Root to shoot ratio

There was a temperature x species interaction ($P<0.01$) in the shoot and root weight. In 'Cefalu' arrowleaf clover, the shoot growth was similar at all temperatures (Figure 6.8a). The root growth of 'Cefalu' arrowleaf clover increased from 0.07 g at 7.9 ^{o}C to 0.10 g at 11.0 ^{o}C, it then decreased with increasing temperature up to 25.4 ^{o}C. For 'Bolta' balansa clover, the shoot growth was the lowest (0.19 g) at 7.9 ^{o}C and highest (0.40 g) at 11.0 ^{o}C (Figure 6.8c). The root growth of 'Bolta' balansa clover increased from 0.08 g at 7.9 ^{o}C to 0.18 g at 11 ^{o}C and then reduced to 0.06 g at the maximum temperature of 25.4 ^{o}C. The shoot growth of 'Prima' gland clover was similar (0.13 g) at temperatures between 7.9 to 15.6 ^{o}C (Figure 6.8c). Its shoot growth declined to 0.05 g at high temperatures between 20.4 to 25.4 ^{o}C. The root weight of 'Prima' gland clover decreased with increasing temperatures from 0.05 g at 7.9 ^{o}C to 0.01 g at 25.4 ^{o}C. In 'Mihi' Persian clover, there was no difference in the root weight under all temperature regimes (Figure 6.8d). The shoot weight was the heaviest (0.37 g) at 25.4 ^{o}C.

These differences between temperature and species in the shoot and root growth caused differences ($P<0.001$) in the root to shoot ratio (Table 6.11). The root to shoot ratio was highest when plants were grown at a constant 11.0 ^{o}C and the lowest at 25.4 ^{o}C air temperature.

Table 6.11 The root to shoot ratio of 'Cefalu'arrowleaf, 'Bolta' balansa, 'Prima'gland and 'Mihi'Persian clovers sown at five constant air temperatures in a controlled environment chamber at Lincoln University in 2012.

Temperature	Root to shoot ratio			
	'Cefalu'arrowleaf	'Bolta' balansa	'Prima'gland	'Mihi'Persian
7.9	0.51 $_{b}$	0.44 $_{b}$	0.41 $_{c}$	0.46 $_{b}$
11	0.57 $_{a}$	0.47 $_{a}$	0.48 $_{a}$	0.49 $_{a}$
15.6	0.46 $_{c}$	0.34 $_{c}$	0.33 $_{d}$	0.40 $_{c}$
20.4	0.51 $_{b}$	0.47 $_{a}$	0.44 $_{b}$	0.50 $_{a}$
25.4	0.28 $_{d}$	0.24 $_{d}$	0.28 $_{e}$	0.28 $_{d}$
	Temperature	Species	Temperature*Species	
P-value	<0.001	<0.001	0.632	
V.r.	50.98	9.98	0.82	
S.E.M.	0.013	0.012	0.026	
L.S.D. (5%)	0.037	0.033	-	

V.r. Variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences. Means with letter subscripts in common are not significantly different at $\alpha = 0.05$.

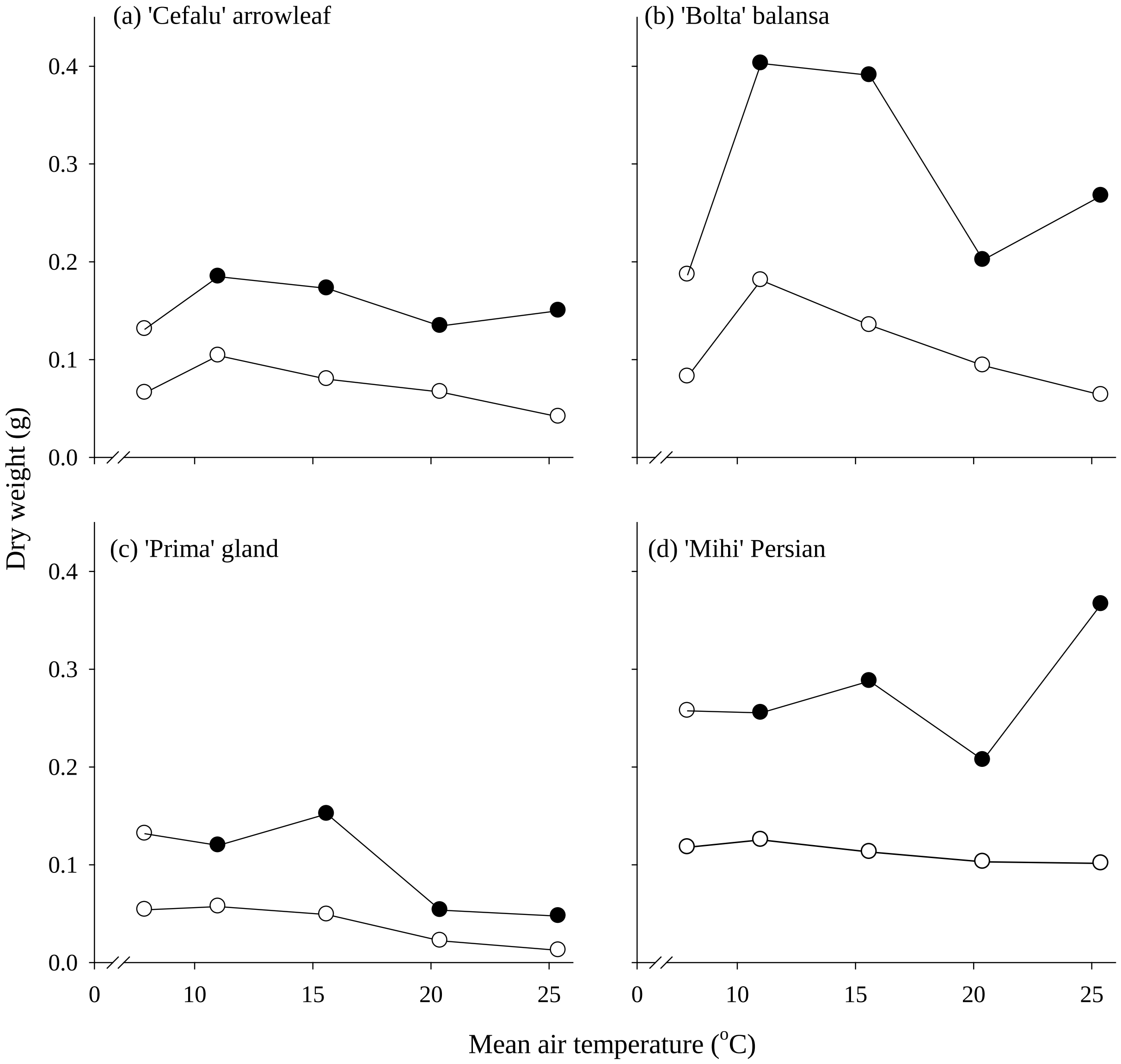


Figure 6.8 The shoot (●) and root (○) weight of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers at different mean air temperatures in a controlled environment chamber at Lincoln University in 2012. Note: The plants had 6-8 leaves on the main stem when they were harvested.

6.3.4 Radiation use efficiency (RUE)

At full flower, the highest crop yield (including weeds) for each species was ~630 g DM/m^2 (SD1) or 6.3 t/ha for ‘Cefalu’ arrowleaf, ~1590 g DM/m^2 (SD3) or 15.9 t/ha for ‘Bolta’ balansa, ~810 g DM/m^2 (SD3) or 8.1 t/ha for ‘Prima’ gland and ~1220 g DM/m^2 (SD3) or 12.2 t/ha for ‘Mihi’ Persian clover (Figure 6.9). These yields required the absorption PAR of ~470 MJ/m^2 in ‘Cefalu’ arrowleaf, ~670 MJ/m^2 in ‘Bolta’ balansa, ~390 MJ/m^2 in ‘Prima’ gland and ~750 MJ/m^2 in ‘Mihi’ Persian clover. Therefore, the RUE was 1.3 (±0.05) g DM/MJ PAR for ‘Cefalu’ arrowleaf, 2.1 (±0.22) g DM/MJ PAR for ‘Bolta’ balansa, 1.6 (±0.26) g DM/MJ PAR for ‘Prima’ gland and 1.3 (±0.13) g DM/MJ PAR for ‘Mihi’ Persian clover (Table 6.12). The percentage of weeds in each plot was shown in Table 6.2.

Table 6.12 Radiation use efficiency (g DM/MJ PAR absorbed) from fitted regression between crop dry matter to full flower (g/m^2) and total PAR absorbed (MJ/m^2) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clover sown on four dates (SD) in 2010 at Lincoln University, New Zealand.

Species	Sowing date (SD)	Radiation use efficiency ± S.E. (g DM/MJ PAR absorbed)	R^2 (%)
‘Cefalu’ arrowleaf	26 Feb	1.26 ± 0.052	98
	24 Mar	1.00 ± 0.037	99
	19 Apr	1.06 ± 0.088	94
	8 Jul	0.97 ± 0.091	91
‘Bolta’ balansa	26 Feb	2.13 ± 0.146	95
	24 Mar	2.54 ± 0.114	99
	19 Apr	2.08 ± 0.221	91
	8 Jul	0.97 ± 0.072	93
‘Prima’ gland	26 Feb	1.39 ± 0.062	98
	24 Mar	1.32 ± 0.125	94
	19 Apr	1.61 ± 0.257	82
	8 Jul	1.04 ± 0.090	93
‘Mihi’ Persian	26 Feb	1.23 ± 0.069	94
	24 Mar	1.46 ± 0.036	99
	19 Apr	1.33 ± 0.126	90
	8 Jul	0.96 ± 0.123	86

SD, sowing date. S.E., standard error. R^2, coefficient of determination.

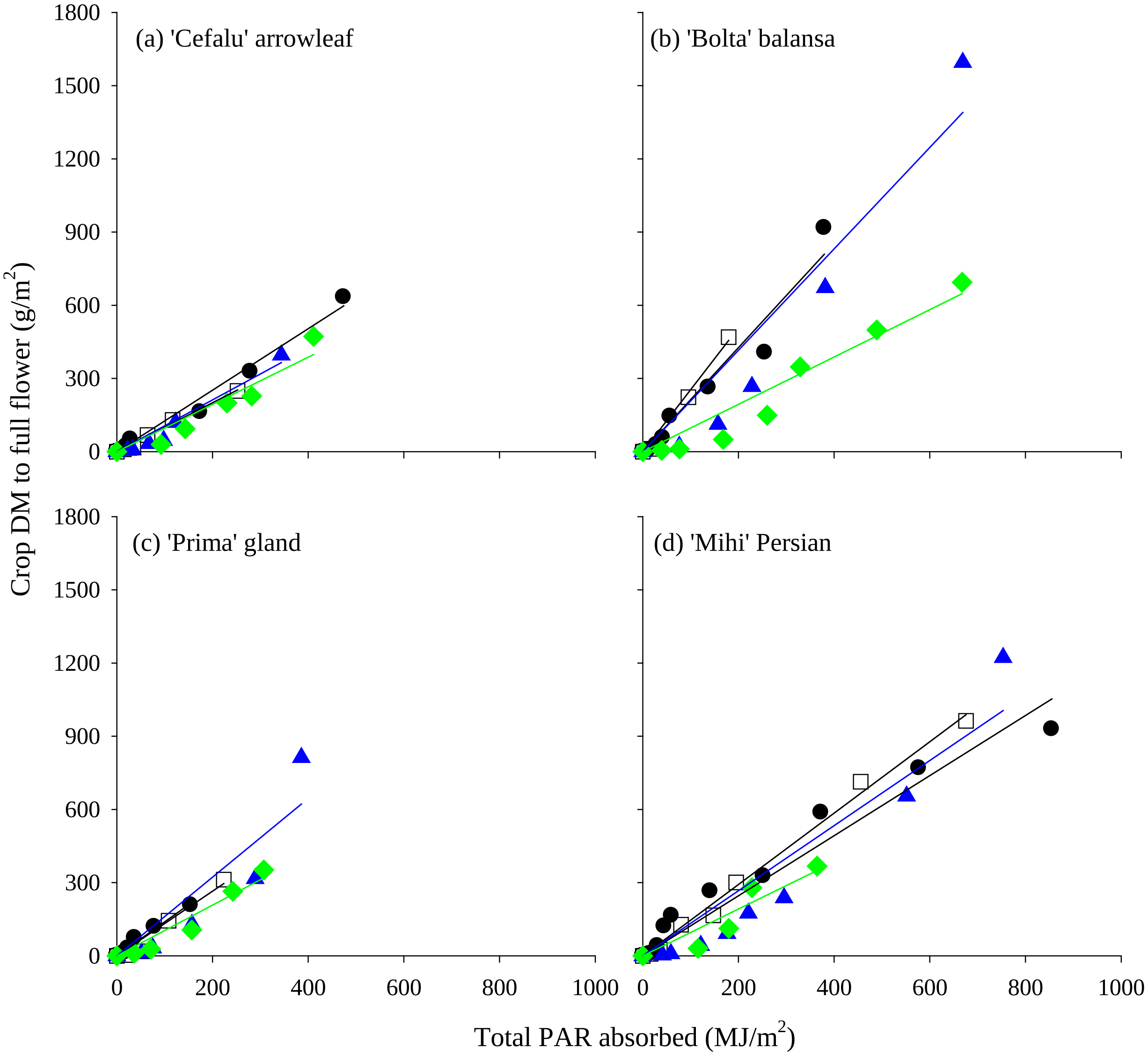


Figure 6.9 Radiation use efficiency (g DM/MJ PAR absorbed) of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆).

6.4 Discussion

6.4.1 Dry matter production

The amount of seed sown in 2010 was sufficient to produce pure swards of legumes and to allow sufficient regeneration of seed to produce similar levels of dry matter production in the second year (2011). The initial sowing rate of 4 kg/ha for 'Bolta' balansa clover produced an average of 11.6 t/ha biomass yield at full flower in the second year which was twice the 5.7 t/ha produced in the initial year (Table 6.3). Similarly, 'Mihi' Persian and 'Prima' gland produced 8.3 t/ha and 2.2 t/ha of dry matter, respectively in 2011 which was also higher than their production in 2010. If these regenerated crops were harvested at physiological maturity rather than at full flower, higher total biomass would be expected than attained in their first year. This shows that 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers were managed successfully for two years and suggests ongoing regeneration could be expected in subsequent years. Thus, these three species show promise for inclusion in the farm systems through the accumulation of seasonal biomass and ability to regenerate in subsequent year (Figure 6.2).

The exception was 'Cefalu' arrowleaf clover which did not regenerate in 2011 to the same level as in 2010 (Figure 6.2). Low productivity in 2011 was attributed to a low population of seedling emergence (up to 11%) and percentage of ground cover (maximum 6%) in the second year. Low population of seedling emergence was mainly due to the high percentage of hardseeds (~97% hardseeds). This is a common situation in arrowleaf clover but can be overcome by introducing heavy grazing during summer to open the sward (Craig and Ballard, 2000). This will result in a wider temperature fluctuations near the soil surface and such environment will likely induce breakdown of hardseeds (Quinlivan, 1965) and has been successfull in Australia. The production of hardseeds and seed softening in annual clovers will be discussed further in Chapter 7. In short, the management for successful regeneration of arrowleaf clover appears more complicated than for the other three species.

Crop yield was dependent on the duration of crop growth and plant population. As expected, crops that had a longer life span (thus longer duration of photosynthesis) and with higher plant populations produced more dry matter. Except for 'Prima' gland clover, SD1 (26 Feb 10) crops which matured late (Figure 6.2) and had the highest plant population (Table 6.8) accumulated the highest yield at full flower (Table 6.3). For example, 'Bolta' balansa clover sown on 26 Feb 10 (SD1) had 60% plant population (Table 6.8) and matured after 2200 ^{o}C d (Figure 6.2b) and produced 10.8 t/ha of DM at full flower (Table 6.3). Conversely, SD4 (8 Jul 10) sown 'Bolta' balansa clover with 30% plant population and a life span of 1670 ^{o}Cd only produced 1.9 t/ha biomass. In 'Prima' gland clover, SD1 produced less dry matter (0.8 t/ha compared with 2.2 t/ha in SD2 (24 Mar 10)) because the crops flowered early in mid-winter (16 Jul 10) and grew slowly due to the lowest temperature of ~7.5 ^{o}C. This suggests the gland clover did not maximize the potential growing season and matured too early to take advantage of all of the available soil moisture.

In 'Cefalu' arrowleaf and 'Mihi' Persian clovers, low total dry matter production in SD2 (24 Mar 10) crops was mainly due to plant populations of less than 22% at emergence (Table 6.8). Plant populations were low because moisture was inadequate in the field. Plots only received 1.8 mm of rainfall throughout the 10 days after sowing, which affected seedling emergence. For SD4 (8 Jul 10) plots, despite receiving 13.0 mm of rainfall, plant populations were considerably low in all species (except for 'Mihi' Persian clover) because of the low soil temperature (mean of 6.2^{o}C) during winter. Similarly, plants grown in the controlled environment chamber at mean soil temperature of 10.9 ^{o}C had the lowest seedling emergence of 29-47% (Figure 5.6). Both SD2 and SD4 plots with low plant population also had the highest weed contents (Table 6.2) because they were not sprayed. In contrast, SD1 plots were sprayed twice with broad leaf and grass weed herbicides (Section 6.2.3) compared with SD2 plots that were only sprayed once with grass weed herbicide. There was no weed control for SD4 plots. As a consequence, weed content in SD1 plots was the lowest. The consequences of weed infestation in both SD2 and SD4 plots would increase competition for resources (nutrient, moisture and light) and affect the measured PAR interception and affect radiation use efficiency calculations (Section 6.3.4). This highlights the importance of weed control because it influences the productivity of annual clovers.

The duration of crop growth differed with the time of sowing (Section 4.4.2). Crops that were sown earlier in autumn at a decreasing photoperiod had a longer duration of vegetative growth and flowered later. In contrast, crops sown on 8 Jul 10 (SD4) into an increasing photoperiod took less time to become reproductive and therefore matured earlier. For example, 'Bolta' balansa clover sown on 26 Feb 10 (SD1) had ~1700^{o}Cd of vegetative growth (27 nodes) compared with ~870 ^{o}Cd (10 nodes) in SD4 (8 Jul 10) before they produced their first bud (Figure 4.3b). This explained the yield differences between these sowing dates. The time of crop maturity and consequently its lifespan is mainly determined by the time of flowering, given the duration from flowering to seed set was predominantly quantified by thermal time (Section 4.4.5). In Chapter 4, the time of flowering was shown to be influenced by the direction and length of photoperiod at seedling emergence (Section 4.4.3). The results in this chapter were consistent with the flowering models developed for each species in Chapter 4 (Figure 4.4) with root mean square deviation (RMSD) of less than 5%.

6.4.2 Canopy expansion and intercepted PAR

Variation in total dry matter among sowing dates was also associated with the timing of canopy closure. In all species, sowing dates that produced high total dry matter reached canopy closure earlier before the crops were mature (Figure 6.4). For example, in ‘Cefalu’ arrowleaf clover, SD1 crops produced the highest yield and reached canopy closure after 2200 ^{o}Cd whereas the other sowing dates with lower yield matured before canopy closure occured (Figure 6.4a). In gland clover, SD3 (19 Apr 10) crops with the highest yield intercepted 95% PAR after 1480 ^{o}Cd, followed by SD2 (1686 ^{o}Cd) and then SD1 (1980 ^{o}Cd) (Figure 6.4c). In all species, SD4 crops produced the lowest yields because they did not reach canopy closure before maturity.

In all species, SD4 crops that established during spring when photoperiod was increasing, had the fastest rate of stem elongation (Figure 6.5 and Figure 6.6) and therefore had the shortest duration of lag phase in dry matter accumulation and canopy growth compared with the autumn sown crops (Table 6.4 and Table 6.6). For example, at 1200 ^{o}Cd, SD4 ‘Bolta’ balansa clover had reached 380 mm height (61 total leaves) compared with 160 mm height (45 total leaves) after the same time period in SD1 (Figure 6.6 and Figure 6.7). Consequently, SD4 plants accumulated their initial 5% of total dry matter much quicker (758 ^{o}Cd versus 1382 ^{o}Cd in SD1) (Table 6.4) and expanded their canopy earlier (878 ^{o}Cd versus 1577 ^{o}Cd in SD1) than SD1 plants (Table 6.6). The acceleration of stem elongation observed in winter sown crops (SD4) was due to warmer temperatures in spring. For example, the internode length of ‘Bolta’ balansa clover was the longest (36.7 mm) at mean temperature of 12.3 ^{o}C (Table 6.9). In contrast, the internode length for autumn sown crops decreased from 30.2 (SD3) to 11.6 mm (SD1) due to falling temperatures from 9.6 to 8.3 ^{o}C. The internode length of these annual clovers was influenced by mean, diurnal and day temperatures (Table 6.9). Similarly, studies on horticulture crops conducted in control environments reported that plant height was influenced by both daily mean temperature and diurnal temperature fluctuations (i.e. differences between day and night temperatures) with longer internode from larger diurnal temperature ranges (Berghage and Heins, 1991; Grimstad and Frimanslund, 1993; Yaping and Heins, 1996). Meanwhile, Erwin *et al*., (1989) found that stem elongation in Easter lily (*Lilium longiflorum* Thunb.) was affected by the differences in diurnal temperature and not mean temperature. According to these researchers, increasing day temperatures increased plant height, whereas increasing night temperature decreased plant height. In contrast, Langton and Cockshull (1997) reported that stem elongation in chrysanthemum (*Chrysanthemum indicum* L.) responded to the absolute day and night temperature rather than diurnal temperature and day temperature appeared to be the dominant factor controlling the internode length.

The long duration of the lag phase in canopy growth for autumn sown crops was attributed to the longer phyllochron at decreasing daylength. For example, the phyllochron of ‘Bolta’ balansa clover sown on 26 Feb 10 (SD1) was the longest, i.e. 78 ^{o}Cd/leaf when seedlings emerged into a 13.7 hour photoperiod. As photoperiod decreased to 10.5 hour in the winter, the phyllochron was shortened to 62 ^{o}Cd/leaf in SD4 (8 Jul 10) crops (Table 6.10). This difference in phyllochron affected the time of first axillary leaf appearance where leaf production begins to accelerate exponentially. Hence, the shorter phyllochron in SD4 crops led to shorter thermal time accumulation to produce their first axillary leaf and consequently reached canopy expansion earlier. These results were consistent with the calibration phyllochron model presented in Figure 3.12 with RMSD of less than 19%. However, regardless of sowing dates, canopy expansion did not begin until spring (Figure 6.10), when temperatures began to rise. Both ‘Bolta’ balansa and ‘Prima’ gland clovers with the shortest phyllochron (62-78 ^{o}Cd/leaf) (Table 6.10) reached canopy expansion within the shortest time (866-1577 ^{o}Cd) (Table 6.6).

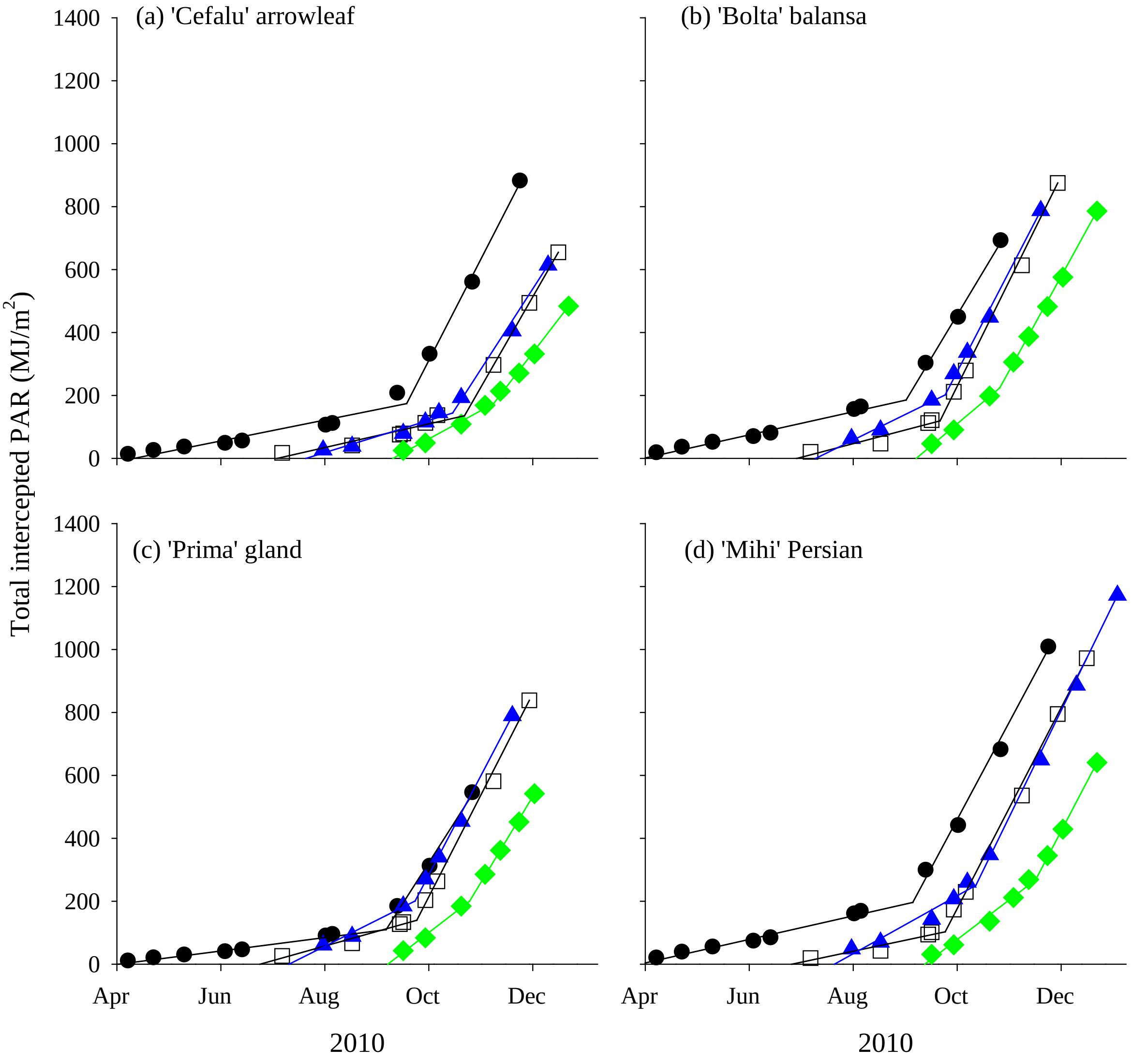


Figure 6.10 Total intercepted PAR versus dates of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (c) 'Prima' gland and (d) 'Mihi' Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). For each fitted line, break point indicates time of canopy expansion.

In all species, sowing dates that produced high total dry matter (Figure 6.2) were associated with a faster rate of canopy expansion (Table 6.6) which led to more PAR intercepted (Figure 6.3). For example, SD1 'Cefalu' arrowleaf crops with the fastest canopy expansion rate of 0.8 MJ m^{-2} ^{o}Cd^{-1} (Table 6.6) intercepted 880 MJ/m^{2} PAR (Table 6.7) to achieve a total yield of 9.4 t/ha within a lifespan of 2590 ^{o}Cd (Figure 6.2a). Conversely, the lowest yield in SD4 crops (2.7 t/ha) was attributed to slowest rate of canopy expansion (0.5 MJ m^{-2} ^{o}Cd^{-1}) thus, lowest total PAR interception (484 MJ/m^{2}) throughout a short lifespan of 1690 ^{o}Cd.

However, the presence of weeds in SD4 crops (44% weeds, Table 6.2) meant the amount of light intercepted by the crop canopy was overestimated because a significant proportion of the total PAR_i probably resulted from the weeds. The same situation occurred in 'Bolta' balansa SD2 (875 MJ/m^2 total PAR_i) and SD4 (786 MJ/m^2 total PAR_i) crops. These had higher total PAR_i than SD1crops (690 MJ/m^2 total PAR_i) (Table 6.7) but this was not reflected in their total crop biomass (15.4 t/ha in SD2 and 4.6 t/ha in SD4 compared with 17.5 t/ha in SD1) (Figure 6.2b). This was because 10-37% of the total dry matter was produced from the weeds and not clovers (Table 6.2). Thus, the corrected total PAR_i from clover is given in Table 6.13 based on the assumption that the dry weight was proportional to the leaf area index.

Table 6.13 Corrected total intercepted PAR (MJ/m^2) by 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland, 'Mihi' Persian clovers after exclusion of PAR_i from the weeds based on the assumption that the dry weight is proportional to the leaf area index.

Sowing date	Total PAR_i (MJ/m^2)			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26 Feb 10	862	669	505	996
24 Mar 10	426	788	654	875
19 Apr 10	442	740	734	1078
8 Jul 10	271	495	369	513

Leaf canopies that intercepted more PAR occured as the outcome of a longer duration and faster rate of canopy expansion. SD1 crops which matured later (2590 °C d in 'Cefalu' arrowleaf, 2200 °C d in 'Bolta' balansa, and 3000°C d in 'Mihi' Persian) had a longer duration to expand their canopy, therefore they intercepted more PAR and contributed to higher dry matter yields. An exception was for SD1 'Prima' gland clover which had longest growth duration (2120 °C d) but yielded less because they flowered too early in mid-winter (1185 °C d) (Figure 6.2c). Thus, gland clover produced less vegetative growth due to unfavourable cold temperatures in the winter which slowed its growth activity. As temperature rose in spring, canopy expansion began but could not compensate for the lost growth because the crop approached maturity and completed its life cycle. For SD4 (28 Jul 10) crops, their thermal time accumulation to reach maturity was the shortest in all species (~1680 °C d in 'Cefalu' arrowleaf and 'Bolta'balansa, 1340 °C d in 'Prima' gland and 1800 °C d in 'Mihi' Persian clover) (Figure 6.2). By the time these crops began a linear canopy expansion, they had already flowered and were near to physiological maturity. Hence, SD4 crops had a shorter duration and slower rate of canopy expansion. Therefore, they intercepted less PAR which resulted in lower dry matter production. These parameters (duration and rate of canopy expansion) accounted for the variation of yield as has been reported on lucerne (Teixeira *et al.*, 2007), soybean (Kumudini *et al.*, 2001), faba bean (De Costa *et al.*, 1997) and potato (Jefferies and Mackerron, 1993) .

6.4.3 Radiation use efficiency (RUE)

Except for ‘Cefalu’ arrowleaf clover, the RUE for winter sown crops (SD4) was lower than autumn sown crops (Table 6.12). This reduction in light conversion efficiency is believed to be attributed to lower temperature (4.3 ^{o}C in mid-July) (Figure 6.1) which slowed the photosynthesis rates of shoots (Figure 6.8). Thus, high root to shoot ratio in annual clovers (Table 6.11) was caused by less shoot growth rather than more root growth. For example, at 7.9 ^{o}C, ‘Bolta’ balansa clover produced the lowest shoot weight (0.19 g) and root weight of 0.08 g which resulted in higher root to shoot ratio (Figure 6.8b). Likewise, the ratio of root to shoot was the lowest for plants grown at 25.4 ^{o}C because they produced more shoot growth. Temperatures between 11.0 and 15.6 ^{o}C were the optimum for shoot growth in ‘Bolta’ balansa clover. Its shoot growth was twice the amount produced by the other three species (Figure 6.8). This implies that at 11.0 – 15.6 ^{o}C, ‘Bolta’ balansa clover photosynthesized at the highest rate which led to the highest production of shoot while there was little change in the root growth. These results, which were obtained from the pot experiment, were found to be consistent with the total biomass production from the field (Figure 6.2).

Biomass partitioning in legumes can be distinguished between the annual and perennial species. Annuals have rapid seedling growth within a short lifespan. Therefore, most of the fixed carbon is allocated for the shoot growth while root growth is relatively small (Campbell *et al.*, 1998; Murray, 2012). Consequently, the root growth in annual species ceases at flowering (Voisin *et al.*, 2003). In contrast, perennial species which have a longer lifespan partition relatively more of their carbon below ground and mobilise root reserves for above ground growth at a later time (Thomas, 2003). The allocation of biomass within perennial species changes with seasonal variation in temperature and photoperiod (Corbel *et al.*, 1999; Moot *et al.*, 2003b). In autumn, falling temperatures led to partitioning of dry matter to below ground growth as crops stock-up reserves for winter survival and subsequent growth in spring

(Hendershot and Volenec, 1993). As temperature rises in spring, crops remobilise assimilates from roots and stolons to shoots (Turner and Pollock, 1998), resulting in stem elongation and canopy expansion. In this present study, it is not known how partitioning of biomass to below ground changes through autumn, winter and spring in annual clovers because roots biomass was not sampled on a continuous basis. Thus, future work could investigate seasonal changes in carbon partitioning on these annual clovers.

RUE among crop species is often reported to be stable over a range of environmental and management variables (Gallagher and Biscoe, 1978; Gifford *et al.*, 1984; Kiniry *et al.*, 1989). Conversely, Justes *et al*. (2000) showed that RUE differed with crop developmental stages in winter oilseed rape (*Brassica napu*s L.). In addition, Sinclair and Muchow (1999) reviewed that RUE may differ with temperature, nitrogen and water availability which influence crop photosynthesis. In this experiment with annual clovers, nitrogen and moisture were not limiting because legume crops have the ability to fix nitrogen and crops were grown throughout autumn to spring where soil moisture was adequate for crop growth. However, crops that were grown at different sowing dates did experience seasonal variations in temperature. Half of the lifespan of SD4 (8 Jul 10) crops was with daily cold temperatures from 4.3^{o}C in mid-July to 15.0 in mid-September (Figure 6.1). Therefore, it is likely that the lower temperatures affected photosynthesis in winter sown crops which reduced their RUE. At these low temperatures, ‘Bolta’ balansa clover had a higher RUE (2.1 g DM/MJ PAR) compared with the other three species (~1.3 g DM/MJ PAR) (Table 6.12). This suggests ‘Bolta’ balansa clover had a higher photosynthesis rate at cool temperatures. In addition, the higher rate of shoot growth from pot experiments at 11.0 and 15.6 ^{o}C (Figure 6.8) supports the idea that ‘Bolta’ balansa clover thrived at cooler temperatures than the other species. The low RUE in SD4 crops may have resulted from weed infestation (Table 6.2) which represented 20-44% of the crop canopy. The RUE for SD4 ‘Bolta’ balansa clover, which had 37% weeds was 0.97 g DM/MJ PAR compared with 2.13 g DM/MJ PAR in SD1 crops which only has 2% weeds (Table 6.12). Based on the low percentage of weeds (1-7%) in the SD1 plots (Table 6.2), the best estimate of RUE is from SD1. The consistenly higher RUE for ‘Bolta’ balansa clover (Table 6.12) helped explain its ability to out yield the other three species (Figure 6.2).

6.4.4 Trade-off between development and growth

This study showed that developmental process (i.e. physiological time) had marked effects (trade-off) on the growth of annual clovers. Although growth and development occur simultaneously, both processes are distinct and driven by different signals. Growth is described as the accumulation of dry matter with time resulted from light interception and photosynthesis activities which led to an increment of plant height, dry matter weight and leaf area expansion (Christian, 1977; Ritchie and NeSmith, 1991) (Section 2.10). Under optimum growth conditions, both growth and development of plants are mainly driven by temperature. Development is defined as the chronological progression through distinct events (phenology); from germination to maturity within a plant life cycle (Section 2.4). Development is also modified by photoperiod.

In annual crops, the critical time in development is flowering because it determines when the crops mature and complete their life cycle. This means that early flowering species will reach physiological maturity earlier. The consequence is that these species will not produce much growth due to their short lifespan. ‘Prima’ gland clover produced the least growth because it flowered as early as in mid-July and had already matured by mid-October when conditions (temperature and moisture) were still favourable for growth (Figure 3.1 and Figure 3.2). Thus, ‘Prima’ gland clover failed to ulitise all of the available growing season. Spring is the most suitable time to maximise dry matter production because of longer daylength (thus, longer duration of photosynthesis), favourable temperatures (day: 15 ^{o}C, night: 6 ^{o}C) and availability of soil moisture. In winter, growth is slow due to low temperatures. Similarly, growth is slow during autumn because of shorter daylengths and falling temperatures. In summer, soil conditions are too dry for growth and the annual clovers are in the form of seeds.

Therefore, successful annual species are those that can grow throughout the spring season and mature in early summer, before drought, to maximize yield. This characteristic can be found in ‘Cefalu’arrowleaf, ‘Bolta’ balansa and ‘Mihi’ Persian clovers which had delayed phenology. ‘Cefalu’ arrowleaf clover flowered at the end of September and matured in mid-December. It produced biomass up to 9.4 t/ha (Figure 6.2a) and had RUE of 1.3 g DM/MJ PAR (Table 6.12). ‘Bolta’ balansa clover produced the highest dry matter up to 17.5 t/ha with RUE of 2.5 g DM/MJ PAR. It flowered in mid-September and matured in mid-November. ‘Mihi’ Persian clover had RUE of 1.2 g DM/MJ PAR and yielded 12.5 t/ha at maturity in the end of December. Based on their ability to produce high yield, these species are worth considering for inclusion in the farm system. This is despite issues of low regeneration due to high percentage of hardseeds in ‘Cefalu’ arrowleaf and low persistence due to softseeds in ‘Mihi’ Persian clover. These issues will be discussed in Chapter 7.

6.5 Conclusions

1. At full flower, accumulated dry matter was up to 6.3 t/ha in ‘Cefalu’ arrowleaf, 15.9 t/ha in ‘Bolta’ balansa, 8.1 t/ha in ‘Prima’ gland and 12.2 t/ha in ‘Mihi’ Persian clover. Total PAR absorbed to accumulate these yields were ~470, 670, 390 and 750 MJ/m^2 respectively. These gave the radiation use efficiency (RUE) of 1.3, 2.1, 1.6 and 1.3 g DM/MJ PAR absorbed, respectively.

2. The regenerated dry matter produced at full flower in 2011 ranged from 1.6 – 2.9 t/ha in ‘Prima’ gland clover and averaged 11.6 t/ha in ‘Bolta’ balansa, 8.3 t/ha in ‘Mihi’ Persian and 0.5 t/ha in ‘Cefalu’ arrowleaf clover across all sowing dates. These second year yields were influenced by the population of seedlings that emerged in the autumn and percentage ground cover throughout the spring.

3. The duration of lag phase in dry matter accumulation and leaf area expansion were influenced by the duration of early seedling establishment. Seedlings that established in the autumn at decreasing photoperiods took longer to elongate their stem and produce their first axillary leaf, which resulted in a prolonged duration of the lag phase. Such crops are vulnerable to winter annual weed invasion.

4. Regardless of sowing dates, rapid canopy expansion did not begin until spring when temperatures began to increase. Thus, crops that were sown late in the winter had a short duration to expand their canopy due to early maturity. In addition, these crops accumulated less biomass due to low winter temperatures and had little time to compensate their biomass in spring before they matured.

7 Seed yield, regeneration and hardseeds breakdown

7.1 Introduction

Annual clovers depend on production of hardseeds to persist over successive generations. Following seed set, their re-establishment depends on the population of seedlings that emerge from the seed bank in subsequent years. This chapter describes the recruitment of seedlings from the seed bank produced from crops initially sown in the autumn and winter of 2010. In addition, the breakdown of hardseeds was assessed in an incubation experiment and as buried seeds for a period of 1.5 years. The time of early seedling emergence and the percentage ground cover achieved are reported as an indicator of success.

The objectives of this chapter were (1) to quantify seed production from four annual clovers sown on four dates, (2) to assess first year regeneration of seedlings in the field from these crops, (3) to determine the pattern of hardseed breakdown of seeds harvested from these crops, and (4) to examine the viability of buried seeds and those dropped on the soil surface over a period of 1.5 years.

7.2 Materials and methods

7.2.1 Seed yield

Seed yield was measured from the dry matter production experiment plots (Chapter 6) at harvest maturity. Harvest maturity was defined as when ≥ 50% of inflorescences within the plot had reached stage 13 for arrowleaf and gland clovers, stage 16 for balansa clover and stage 12 for Persian clover based on their reproductive development scale (Table 4.1 to Table 4.4). The dates of seed harvest for each species are shown in Table 7.1. An area of 1m^2 was harvested from each subplot and cut to ground level with hand shears. The biomass (reproductive and vegetative components) was air dried in a crop laboratory, weighed and passed through a fixed Kurpeltz thresher to separate the seeds from plant material. The seeds collected were then hand-cleaned to remove other materials such as sticks and leaves, dirt, stones and weed seeds that were involuntarily collected with the harvested seeds. The clean seeds were weighed to determine yield. At the same time, the weight of 1000 seeds was measured and individual seed weight was reported. Harvest index, defined as the ratio of seed to plant weight was calculated from Equation 7.1:

Equation 7.1 $$\text{Harvest index (\%)} = \frac{Dried\ seed\ weight\ (g)}{Dried\ total\ plant\ weight\ (g)}\ x\ 100$$

Table 7.1 The date of seed harvest for 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Species			
(SD)	'Cefalu' arrowleaf	'Bolta' balansa	'Prima' gland	'Mihi' Persian
26 Feb	10 Jan 11	25 Nov 10	25 Nov 10	18 Jan 11
24 Mar	11 Jan 11	14 Dec 10	8 Dec 10	18 Jan 11
19 Apr	11 Jan 11	16 Dec 10	16 Dec 10	25 Jan 11
8 Jul	17 Jan 11	2 Jan 11	16 Dec 10	25 Jan 11

7.2.2 Seedling regeneration

Seedling regeneration was observed from the dry matter production experiment plots sown on four dates in 2010 (Chapter 6). The plots were ungrazed throughout the experimental period to allow maximum flowering and seed set. On 29 April 2011, the dead herbage was removed using a rotary mower to facilitate seedling establishment. The date of the earliest seedling emergence within a subplot was recorded. No seedling count was taken in the experimental plots, but germination tests (Section 7.2.3), buried and sown seed experiments (Section 7.2.4) were conducted from the harvested seeds to assess the pattern of hardseed breakdown and seed viability in relation to time, to signify its regeneration potential. The plots were sprayed on 1 August 2011 with Gallant Ultra (a.i. 520 g/L haloxyfop-P) at 250 mL/ha to control grass weeds. The experimental area remained ungrazed and clover seedlings were left to grow to complete their life cycle. The percentage cover of regenerated clovers was visually assessed on 22nd September, 7th October, 25th October and 2nd November 2011.

7.2.3 Hardseed breakdown

Germination tests were done at three month intervals from end of March 2011 to April 2012 and six months later in October 2012 to investigate the breakdown of hardseeds throughout an 18 month period. The experiment used arrowleaf, balansa, gland and Persian clover seeds that were harvested (Section 7.2.1) from the dry matter production experiment plots sown on four sowing dates in 2010. These seeds were stored in sealed plastic bottles and kept at room temperature to approximate on-farm storage. Fifty seeds from each of the seed lots were placed on a wetted blotting paper in sealed plastic containers and germinated at 19.7 ^{o}C for both arrowleaf and balansa clover, 16.2 ^{o}C for gland clover and 32.6 ^{o}C for Persian clover, for two days. These temperatures and duration were selected based on their optimum temperature and thermal time requirement from the results of the previous germination experiment (Table 3.4). No scarification or pre-conditioning treatment was applied to the seeds prior to germination tests. The percentage of germinated seeds was plotted against time (months) to determine the pattern of hardseed breakdown when in ambient storage.

7.2.4 Buried and sown seeds experiment

This experiment used seeds harvested from the annual clovers sown on 24 March 2010. This sowing date was chosen because it had produced the highest seed yield (Table 7.2). The experiment was a completely randomized design that comprised the four clover species under five burial durations (3, 6, 9, 12, and 18 month) and three replicates. A set of 60 polyvinyl chloride (PVC) tubes (250 mm length, 60 mm diameter) were filled with non-sterilised soil collected from the area surrounding the location where the clovers were previously grown in Iversen 9 field, Lincoln University. During this process, a seed bag (F57 filter bag, 25 µm porosity, ANKOM Tech.) containing 200 clover seeds was buried in each tube at 60 mm from the bottom end of the tube. The seed bag was then covered with the remaining soil up to 20 mm at the top end of the tube. The top 20 mm of the tube was left free of soil (Figure 7.1). On the 12 month tubes, an additional 200 seeds were placed on the soil surface, layered with a fine nylon mesh to prevent the seeds from sinking into the soil. On 11 April 2011, all 60 tubes were positioned vertically in a 10 x 6 matrix, 100 mm apart in Iversen 9 field, Lincoln University (Plate 16).

At 3, 6, 9, 12, and 18 months, bags were removed from the tubes and germinated seeds were counted. At the same time, emerged seedlings were counted and removed from the top of the 12 month tubes. This method was chosen on the basis of work reported by James and Rahman (2003). Monitoring of soil temperature was conducted at different depths throughout 2011 (Figure 7.2).

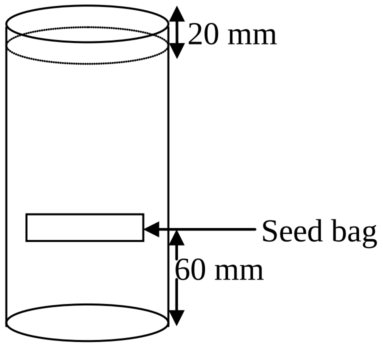


Figure 7.1 Diagram of burried seed bag at 60 mm depth from the bottom of PVC tube and covered with soil up to 20 mm at top end.

Plate 16 The remaining 48 tubes on 1st August 2011 at Iversen 9 field, Lincoln University. Note: Remaining tubes were re-randomized at every removal time.

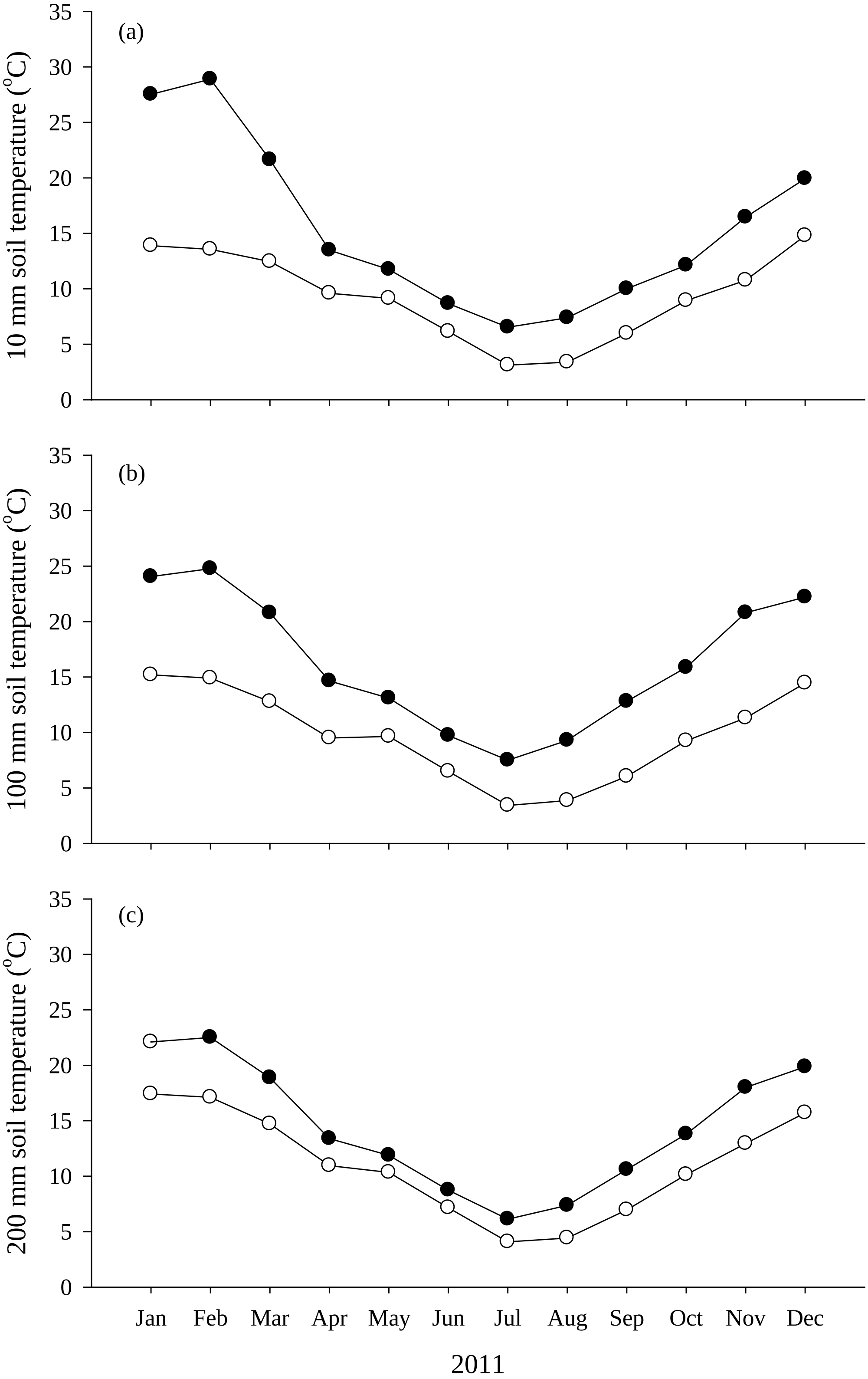


Figure 7.2 Average maximum (●) and minimum (○) soil temperatures at (a) 10 mm, (b) 100 mm, and (c) 200 mm depth in Iversen 9 field, Lincoln University, Canterbury, New Zealand in 2011.

7.2.5 Data analysis

Statistical analysis used Genstat 12.2 (Lawes Agricultural Trust). All variates were analyzed using analysis of variance (ANOVA) procedures in a split-plot (seed yield and seedling regeneration experiments) and complete randomized (hardseed breakdown and buried seeds experiments) design structure. Treatment means were compared by Fisher's protected least significant difference (l.s.d.) test whenever the ANOVA indicated that differences among treatments presented $P<0.05$. Pooled standard errors of the mean were reported for each measured variable.

7.3 Results

7.3.1 Seed yield

The seed yield was influenced by a sowing date x species interaction (Table 7.2). Sowing on 26th February 2010 yielded the most seed for ‘Cefalu’ arrowleaf clover. In ‘Bolta’ balansa and ‘Prima’ gland clovers, the 24th March 2010 sowing date produced the greatest seed yield.

Table 7.2 Seed yield (kg/ha) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date	Seed yield (kg/ha)			
(SD)	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	914	2023	1295	823
24 Mar	683	2309	2370	847
19 Apr	709	859	1646	723
8 Jul	221	1375	536	862
	SD	Species	SD*Species	
P- value	<.001	<.001	<.001	
V.r.	14.5	34.4	7.3	
S.E.M.	91.2	83.6	171.1	
Except when comparing means at the same SD			167.2	
L.S.D. (5%)	291.8	239.7	488.0	
Except when comparing means at the same SD			479.4	

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

7.3.2 Harvest index

There was also an interaction (P<0.007) between sowing date and species for the harvest index (Table 7.3). In ‘Cefalu’ arrowleaf and ‘Mihi’ Persian clover, crops sown on 8 Jul 10 (SD4) gave the highest harvest index of 19.7% and 21.2% respectively. For ‘Bolta’ balansa and ‘Prima’ gland clovers, their maximum harvest indices of 33.9% and 32.4% were from SD2 (24 Mar 10) crops.

Table 7.3 Harvest index (%) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date (SD)	Harvest index (%)			
	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	9.2	18.6	19.3	11.2
24 Mar	15.6	33.9	32.4	14.4
19 Apr	17.0	13.3	25.0	15.5
8 Jul	19.7	24.2	28.2	21.2

	SD	Species	SD*Species
P- value	<0.003	<.001	<0.007
V.r.	9.87	17.22	3.11
S.E.M.	1.45	1.29	2.66
Except when comparing means at the same SD			2.58
L.S.D. (5%)	4.64	3.70	7.60
Except when comparing means at the same SD			7.40

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

7.3.3 Individual seed weight

The individual seed weight was also affected by the sowing date x species interaction (Table 7.4). An average seed weight of 1.24 mg was found in ‘Cefalu’ arrowleaf and 1.12 mg in ‘Bolta’ balansa clovers across all sowing dates. Individual seed weight ranged from 0.88 to 1.01 mg for ‘Prima’ gland and from 1.56 to 1.74 mg for ‘Mihi’ Persian clover.

Table 7.4 Individual seed weight (mg) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown on four dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

Sowing date (SD)	Individual seed weight (mg)			
	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26 Feb	1.27	1.15	0.94	1.74
24 Mar	1.21	1.11	1.01	1.56
19 Apr	1.25	1.11	1.00	1.64
8 Jul	1.22	1.12	0.88	1.59

	SD	Species	SD*Species
P- value	0.042	<.001	0.014
V.r.	4.133	403.377	2.774
S.E.M.	0.016	0.014	0.029
Except when comparing means at the same SD			0.029
L.S.D. (5%)	0.051	0.041	0.084
Except when comparing means at the same SD			0.082

V.r. variance ratio; S.E.M. Standard error of the mean; L.S.D., Least significant differences.

7.3.4 Seedling regeneration

Rainfall on 28th December 2010 of 28 mm led to the earliest seedling emergence between 2nd and 13th January 2011 for 'Bolta' balansa clover and between 12th and 22nd January 2011 for 'Prima' gland clover (Figure 7.3). Based on germination tests (26th March 2011) of the harvested seeds, these emerged seedlings were estimated to represent about 13% of the seed set in 'Bolta' balansa and 3% in 'Prima' gland clover. 'Cefalu' arrowleaf clover seeds did not emerge until after 18.0 mm rainfall between 5th and 7th March 2011. The emerged seedlings between 10th and 20th March 2011 constituted about 3% of the seed population. In contrast, ~96% of 'Mihi' Persian clover seedlings emerged on 23rd March 2011 following continuous rainfall and drizzle (13.6 mm) between 15th and 22nd March 2011.

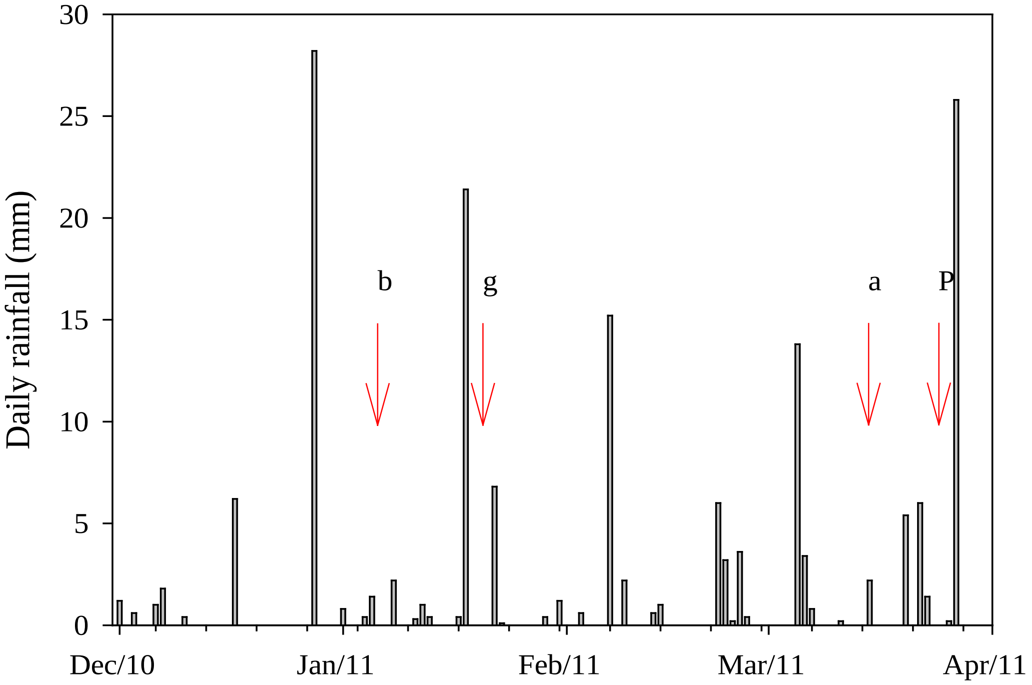


Figure 7.3 Daily rainfall from Broadfield Meteorological Station (2 km north of the site), Canterbury, New Zealand. Arrows indicate time of earliest seedling emergence of (a) 'Cefalu' arrowleaf, (b) 'Bolta' balansa, (g) 'Prima' gland and (P) 'Mihi' Persian clover.

7.3.4.1 Regeneration cover (%) within 42 m^2 subplots

The development and re-growth of these regenerated annual clovers from March to November 2011 are shown in Plate 17 to Plate 20. There were differences ($P<0.05$) among the sowing dates in seedling regeneration cover within the 42 m^2 subplots (Figure 7.4). In 'Bolta' balansa clover, regeneration cover ranged from 61 (±8.3) % in SD1 (26 Feb 10) to 91 (±2.6) % in both SD2 (24 Mar 10) and SD3 (19 Apr 10) on 25th October 2011 (Figure 7.4b). 'Prima' gland clover had final regeneration cover between 30 (±9.6) % (SD1) and 65 (±7.4) % (SD2) (Figure 7.4c). For 'Cefalu' arrowleaf and 'Mihi' Persian clovers, their regeneration cover averaged 6 (±1.4) % and 17 (±2.3) % respectively across all sowing dates.

(a) 23rd March 2011 (b) 9th September 2011

(c) 11th October 2011 (d) 2nd November 2011

Plate 17 Regeneration of 'Cefalu' arrowleaf clover from 23rd March to 2nd November 2011 in Iversen 9 field, Lincoln University, New Zealand.

(a) 9th March 2011

(b) 9th September 2011

(c) 11th October 2011

(d) 2nd November 2011

Plate 18 Regeneration of ‘Bolta’ balansa clovers from 9th March to 2nd November 2011 in Iversen 9 field, Lincoln University, New Zealand.

(a) 10th March 2011 (b) 9th September 2011

(c) 11th October 2011 (d) 2nd November 2011

Plate 19 Regeneration of ‘Prima’ gland clovers from 10th March to 2nd November 2011 in Iversen 9 field, Lincoln University, New Zealand.

(a) 23rd March 2011

(b) 9th September 2011

(c) 11th October 2011

(d) 2nd November 2011

Plate 20 Regeneration of ‘Mihi’ Persian clovers from 23rd March to 2nd November 2011 in Iversen 9 field, Lincoln University, New Zealand.

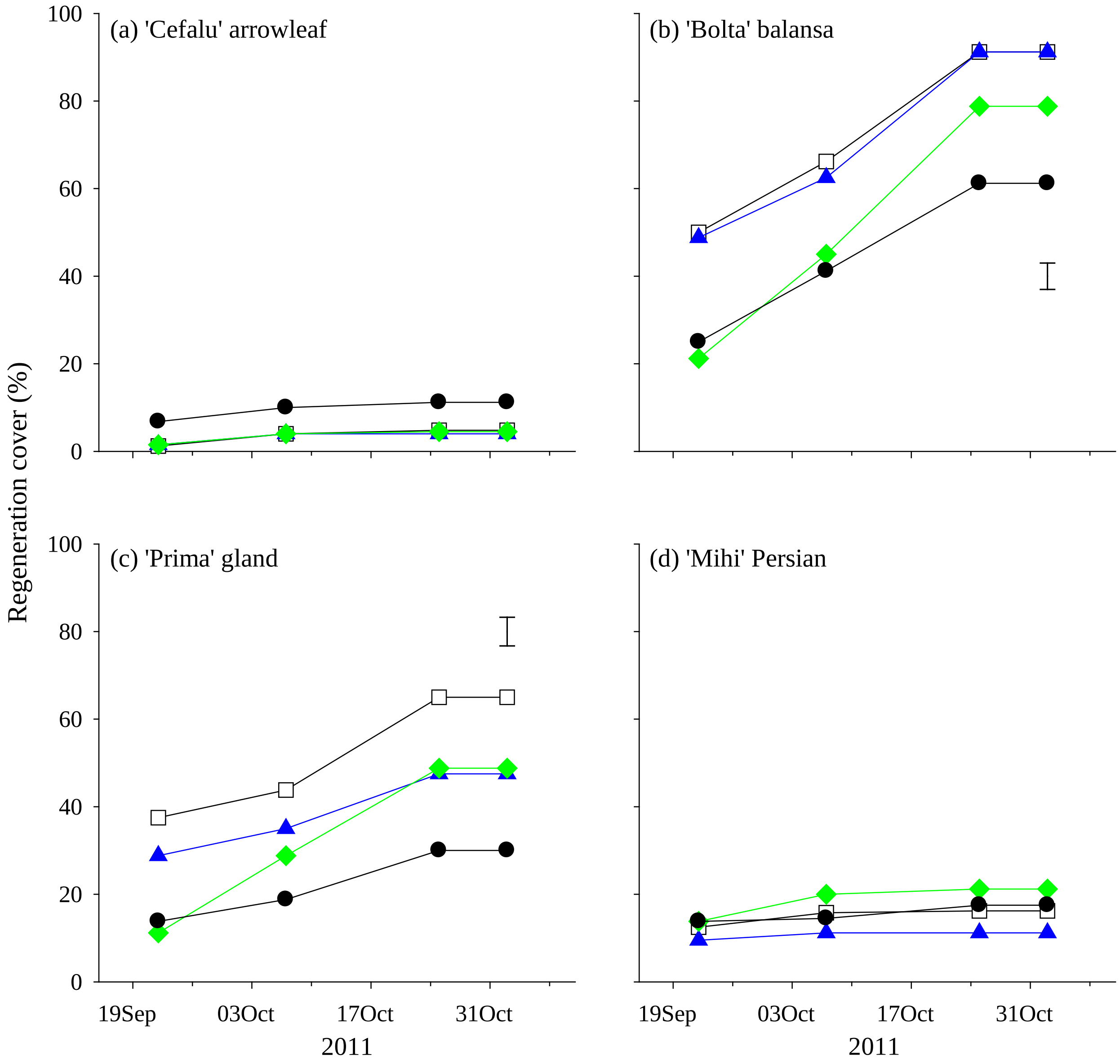


Figure 7.4 Visual assessment of the first year regeneration cover (%) from 22nd September to 2nd November 2011 of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (☐), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). Bars represent one standard error of the mean at the final point where sowing dates were different (P<0.05). Assessment between March and September are not reported because they are insignificant.

7.3.5 Hardseeds breakdown

7.3.5.1 ‘Cefalu’ arrowleaf

The breakdown of hardseeds, represented by the germination percentage increased ($P<0.05$) throughout the 22 month period following seed harvest (Figure 7.5a). The relationship between the seed germination and time (month) after seed harvest was described by linear (SD1) or asymptotic exponential (SD2-SD4) functions (Table 7.5). The seeds harvested from four sowing dates showed different germination potential. The maximum germination ranged from 18.6% in SD1 (26 Feb 10) to 56.0% in SD3 (19 Apr 10) after 22 months from seed harvest.

7.3.5.2 ‘Bolta’ balansa

The germination increased quadratically ($P<0.05$) to a maximum of 30.0% (17.5 months after harvest) for SD2 and 35.9% (16.6 months after harvest) for SD4 (Figure 7.5b). It then started to decrease thereafter. The coefficients of fitted relationship for all sowing dates are given in Table 7.5.

7.3.5.3 ‘Prima’ gland

The relationship between germination and time was either a quadratic or linear function (Figure 7.5c, Table 7.5). For seeds harvested from SD3 and SD4, germination only increased up to 12.2% on 23 months following seed harvest.

7.3.5.4 ‘Mihi’ Persian

The seeds of ‘Mihi’ Persian clover were all soft seeds. Throughout the period of 21 months after harvest, germination averaged 95.2% across all sowing dates (Figure 7.5d).

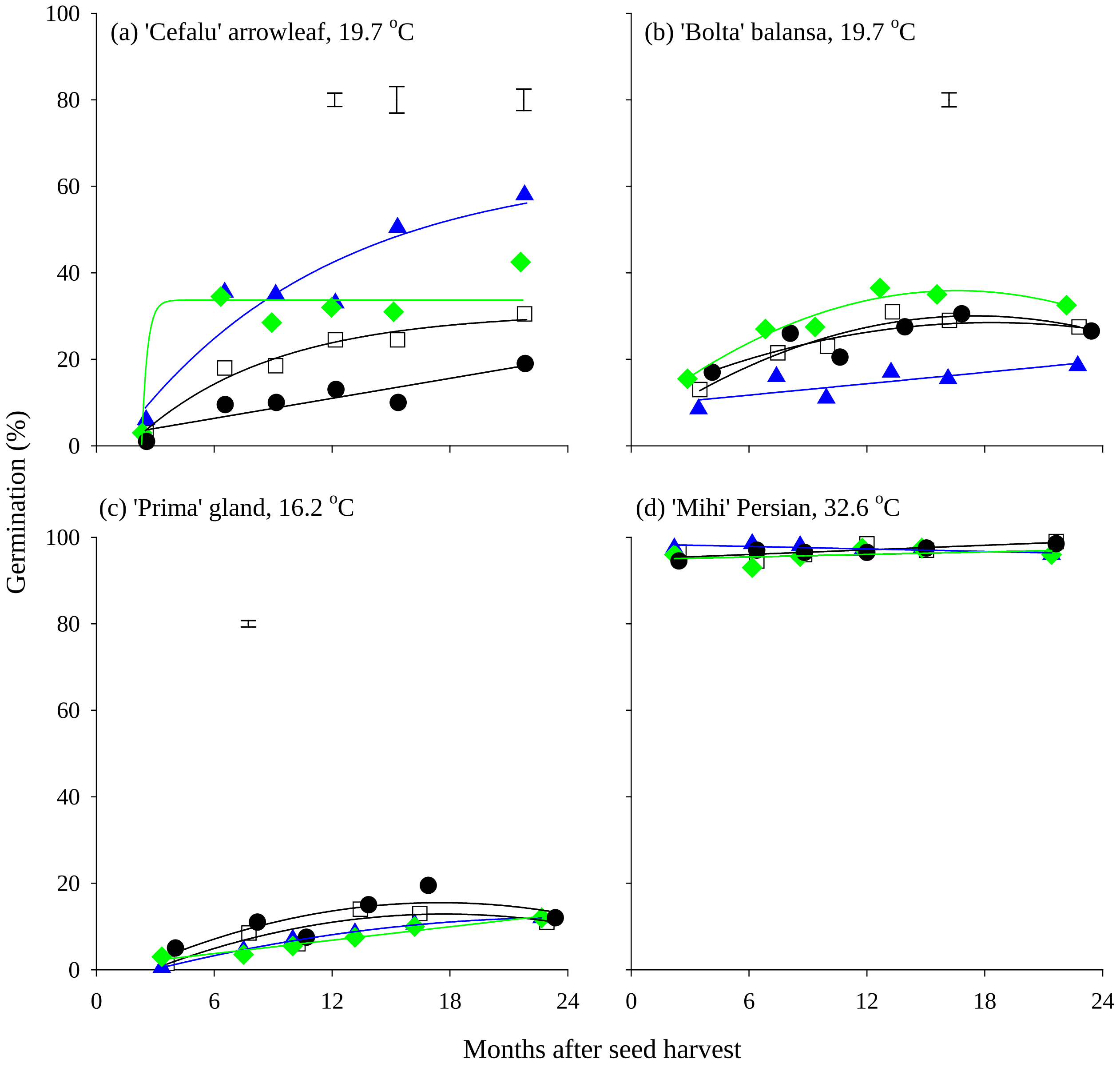


Figure 7.5 Germination (%) of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clovers sown on four dates (SD) at Lincoln University, Canterbury, New Zealand. SD1: 26 Feb 10 (●), SD2: 24 Mar 10 (□), SD3: 19 Apr 10 (▲), SD4: 8 Jul 10 (◆). Bars represent one standard error of the mean where sowing dates were different (P<0.05). For dates of seed harvest, see Table 7.1.

Table 7.5 Coefficients of fitted relationship between germination (%) and time (months) after seed harvest in four sowing dates (SD) of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers.

Species	SD	Relationship	R^2 (%)	P-value
'Cefalu' arrowleaf	1	$y = 1.72 + 0.771x$	74	0.015
	2	$y = 30.91 – 39.11(0.8674)^x$	94	0.007
	3	$y = 64.80 – 71.20 (0.9084)^x$	81	0.040
	4	$y = 33.70 – 122718 (0.0286)^x$	79	0.046
'Bolta' balansa	1	$y = 9.86 + 2.03x - 0.055x^2$	49	0.172
	2	$y = 3.02 + 3.09x – 0.089x^2$	91	0.013
	3	$y = 9.09 + 0.439x$	51	0.068
	4	$y = 6.24 + 3.58x – 0.108x^2$	91	0.012
'Prima' gland	1	$y = -3.99 + 2.23x – 0.064x^2$	44	0.197
	2	$y = -5.32 + 2.05x – 0.058x^2$	70	0.078
	3	$y = -3.49 + 1.28x – 0.026x^2$	100	<.001
	4	$y = 0.64 + 1.517x$	95	<.001
'Mihi' Persian	1	$y = 94.82 + 0.174x$	73	0.018
	2	$y = 94.95 + 0.178x$	41	0.102
	3	$y = 98.46 - 0.096x$	47	0.081
	4	$y = 94.86 + 0.098x$		0.431

SD, sowing date. R^2, coefficient of determination. For dates of sowing, see Table 7.1.

7.3.6 Germination of the buried seeds

The response of seed germination to burial duration was described by a quadratic function (Figure 7.6; Table 7.6). After three months burial, the percentage of seeds germinated inside the bag was 1.8% for 'Cefalu' arrowleaf, 4.3% for 'Bolta' balansa, 5.5% for 'Prima' gland and 0.5% for 'Mihi' Persian clovers. Burial duration > 3 months resulted in a quadratic decline ($P=0.015$) in germination of 'Prima' gland clover seeds. For 'Mihi' Persian clover seeds, germination ceased beyond the three month burial and all seeds appeared to have rotted.

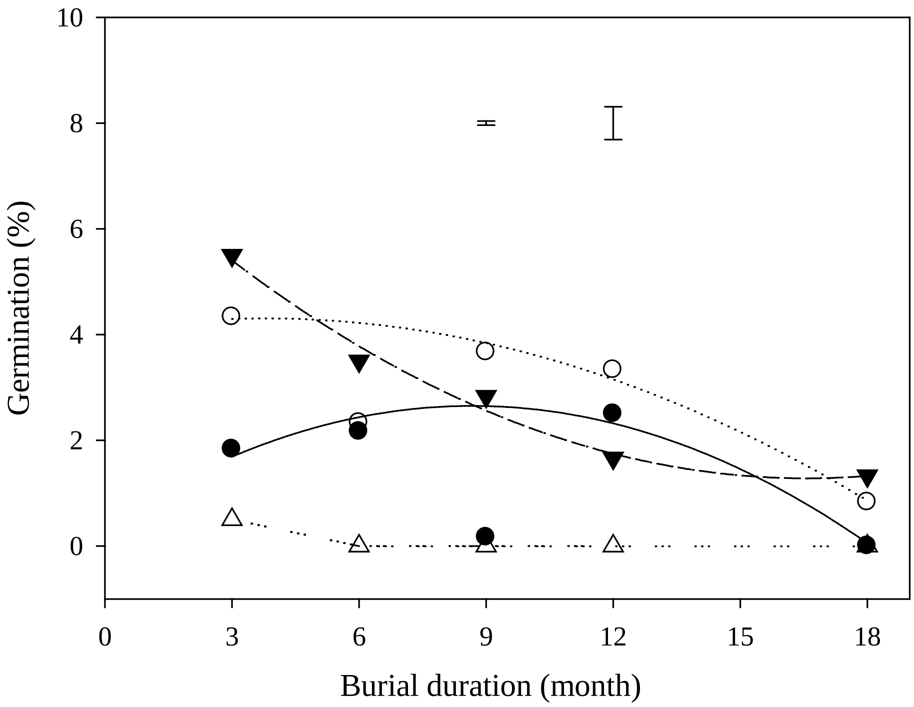


Figure 7.6 Germination of 'Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) clover seeds buried at 170 mm depth at Iversen 9 field, Lincoln University, New Zealand. Bars represent one standard error of the mean where species were different ($P<0.05$).

Table 7.6 Coefficients of fitted relationship between germination (%) and burial duration (month) in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover seeds.

Species	Relationship	R^2 (%)	P-value
'Cefalu' arrowleaf	$y = 0.41 + 0.52x – 0.030x^2$	88	0.186
'Bolta' balansa	$y = 4.07 + 0.13x – 0.017x^2$	97	0.096
'Prima' gland	$y = 7.43 - 0.74x + 0.022x^2$	97	0.015
'Mihi' Persian	No relationship was fitted		

R^2, coefficient of determination.

7.3.7 Survival of the buried seeds

The survival of seeds declined asymptotically (P=0.001) with burial duration for both ‘Cefalu’ arrowleaf and ‘Prima’ gland clovers (Figure 7.7; Table 7.7). For ‘Bolta’ balansa clover, the percentage of seeds recovered inside the bag decreased quadratically (P=0.016) to 16.1% after 12-month burial. From the beginning to the end of the 18-month burial duration, ‘Cefalu’ arrowleaf had the most seeds survived (Figure 7.7). In this experiment, the loss of seeds was accounted for by germination and decay. For ‘Mihi’ Persian clover, there was no seed survival throughout the burial duration with all seeds decayed (Plate 21d).

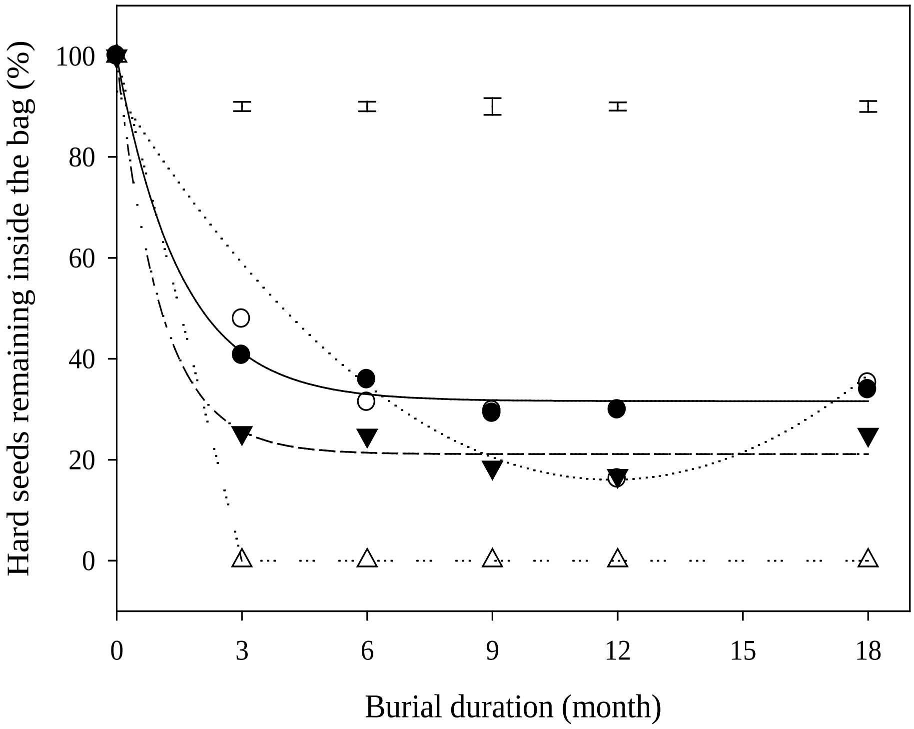


Figure 7.7 Survival of ‘Cefalu’ arrowleaf (●), ‘Bolta’ balansa (○), ‘Prima’ gland (▼) and ‘Mihi’ Persian (△) clover seeds buried at 170 mm depth at Iversen 9 field, Lincoln University, New Zealand. Bars represent one standard error of the mean where species were different (P<0.05).

Table 7.7 Coefficients of fitted relationship between survived seeds (%) and burial duration (month) in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clover seeds.

Species	Relationship	R^2 (%)	P-value
‘Cefalu’ arrowleaf	$y = 31.62 + 68.34(0.521)^x$	99	<.001
‘Bolta’ balansa	$y = 93.03 – 13.0x + 0.549x^2$	90	0.016
‘Prima’ gland	$y = 21.13 + 78.86(0.387)^x$	98	0.001
‘Mihi’ Persian	No relationship was fitted		

R^2, coefficient of determination.

(a) ‘Cefalu’ arrowleaf (b) ‘Bolta’ balansa

(c) ‘Prima’ gland (d) ‘Mihi’ Persian

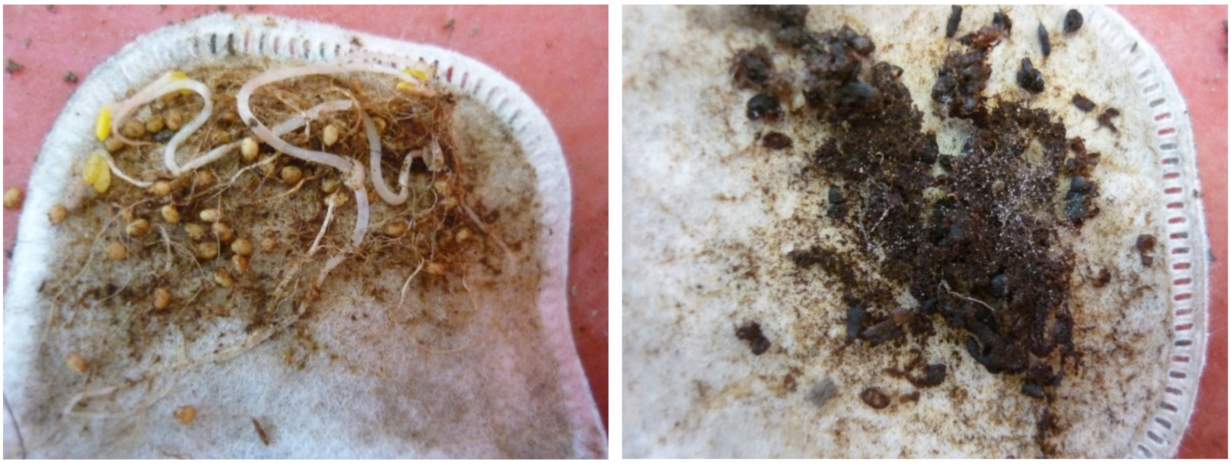


Plate 21 Germination and recovery of buried seeds of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’gland and (d) ‘Mihi’ Persian clovers on 12 April 2012 (after 12-month burial duration, at 170 mm depth).

7.3.8 Seedling emergence

On 22 July 2011, i.e. three months after seeds were sown; 'Bolta' balansa had the highest seedling emergence of 23.3%, followed by 'Mihi' Persian (19.2%), 'Prima' gland (13.5%) and 'Cefalu' arrowleaf (10.3%) (Figure 7.8). In all species, there was no significant increment in the seedling emergence thereafter. From the period of 22 July 2011 (3-month) to 12 April 2012 (12-month), the mean percentage of emergence was 24.9% for 'Bolta' balansa, 19.2% for 'Mihi' Persian, 15.1% for 'Prima' gland and 10.9% for 'Cefalu' arrowleaf clover.

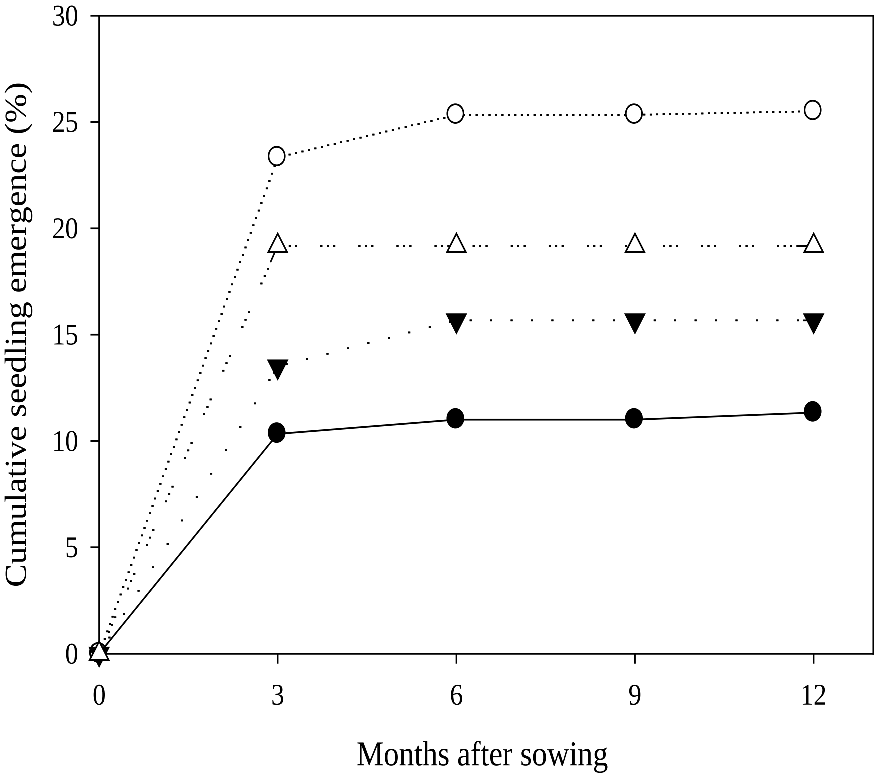


Figure 7.8 Cumulative emergence of 'Cefalu' arrowleaf (●), 'Bolta' balansa (○), 'Prima' gland (▼) and 'Mihi' Persian (△) clover from the soil surface of buried tubes over 12-months at Iversen 9 field, Lincoln University, New Zealand.

7.4 Discussion

7.4.1 Seedling recruitment

Based on germination tests (26th March 2011) of the harvested seeds, approximately 13% of ‘Bolta’ balansa and 3% of ‘Prima’ gland clover seeds that dropped on the ground emerged following summer rainfall in January 2011 (Figure 7.3,Figure 7.5). About 3% of ‘Cefalu’ arrowleaf and 96% of ‘Mihi’ Persian clover seeds were estimated to emerge in March 2011. These estimations were based on controlled laboratory experiment where the seeds were exposed to their optimum condition. This means that the actual percentage of seedling emergence in the field could be much lower than estimated.

In July 2011, 23% of ‘Bolta’ balansa, 14% of ‘Prima’ gland and 10% of ‘Cefalu’ arrowleaf seedlings in the field had emerged (Figure 7.8). In contrast, ‘Mihi’ Persian clover that was predicted to produce 96% emergence in March (laboratory experiment) only had 19% field emergence in July (Figure 7.8). The field emergence of ‘Mihi’ Persian clover did not reflect either germination (Figure 7.5d) or emergence (Figure 5.6d) results from experiments that were conducted in controlled environments. The low seedling emergence in ‘Mihi’ Persian clover in the field was also encountered during field establishment in 2010 (Table 6.8). Specifically, plots from SD2 (24 Mar 2010) produced only 22% seedling emergence from the number of seeds sown. This was similar to the 19% emergence obtained in this experiment. Furthermore, this experiment also used seeds produced from the SD2 plots (Section 7.2.4). This result showed that although ‘Mihi’ Persian clover seeds are soft and had high germination when tested in the laboratory, their field establishment was low.

On 25th October 2011, ‘Bolta’ balansa clover recorded the highest regeneration cover of 91% within the 42 m^2 subplot (Figure 7.4b). With only 23% of plant population in July, ‘Bolta’ balansa clover was able to expand to 91% ground cover in just three months. ‘Prima’ gland clover produced 65% ground cover (Figure 7.4c) from 14% seedling recruitment. For ‘Cefalu’ arrowleaf and ‘Mihi’ Persian clovers, their 10% and 19% seedling recruitment respectively were insufficient to establish a minimum of 50% ground cover. Both ‘Cefalu’ arrowleaf and ‘Mihi’ Persian clovers only managed to cover 6% and 17% of the 42 m^2 area respectively (Figure 7.4a,d). The slow development rate (i.e. leaf appearance (Figure 3.12a) and time to axillary leaf production (Table 3.9) of ‘Cefalu’ arrowleaf clover may contribute to their inability to compete in a sward. ‘Cefalu’ arrowleaf clover was unable to maintain an acceptable ground cover. For ‘Mihi’ Persian clover, many of their matured seeds were not shed on the ground because of their inflated calyces in the inflorescences (Plate 22). Upon contact with moisture, these seeds germinated and seedlings emerged from within the inflorescences that were still attached to the dead parent plant. Unless these inflorescences were set on the ground, the chances of the emerged seedlings surviving were minimal. On 29th April 2011, the dead herbage was removed by a rotary mower in an attempt to facilitate seedling establishment (Section 7.2.2). Because most of the ‘Mihi’ Persian seeds were still attached to the parent plant, the mowing activity racked-off the herbage together with the majority of the seeds and minimized the seed population. Only those that were left on the ground survived. This process explained the poor regeneration and establishment of ‘Mihi’ Persian clover.

Plate 22 Inflated calyces and emerged cotyledons of ‘Mihi’ Persian clover on 23rd March 2011 from the inflorescences that were still attached on the dead parent plant

In the middle of the second autumn (April 2012), there was still 204 kg/ha of ‘Cefalu’ arrowleaf, 374 kg/ha of ‘Bolta’ balansa and 398 kg/ha of ‘Prima’ gland clover hard seeds left in the soil (Figure 7.7). These represented more than 30 times the amount of seeds initially sown. These results showed that these three annual clovers produced a huge population of hard seeds which could be managed for regeneration in subsequent years. The persistence of a legume is dependent on the production of hard seeds (Norman *et al.*, 1998; Quinlivan, 1971) because it distributes germination across several seasons (Bewley and Black, 1985). Therefore, observation of seedling regeneration in the second and third years would be required to determine the seedling recruitment pattern and seed bank dynamics. The outcome could be used to develop management options to keep an on-going population of these species in the field. Of particular relevance is the frequency with which there is a need to re-sow or allow set seed. For ‘Mihi’ Persian clover, all seeds were soft and none were recovered in the soil after three months burial (Figure 7.7). This means that ‘Mihi’ Persian clover did not persist because it did not produce any hardseed. Successful establishment of this species would depend on re-sowing every year unless a cultivar with a greater percentage of hardseed was obtained. The utilisation of soft seeded ‘Mihi’ Persian clover would be suitable for grazing, hay cut and stripped for seed annually, rather than as an ongoing component in a sward (Lee and Reed, 1993)

To improve seedling regeneration of annual clovers, the pasture needs to be grazed intensively during summer to remove the top growth and open up the sward (Craig and Ballard, 2000; Monks *et al.*, 2008). The clearance of pasture during summer will result in wider temperature fluctuations near the soil surface which could increase the rate of hardseed breakdown (Quinlivan, 1965). A proportion of seeds consumed by grazing livestock may also survive passage through the animal to be returned to the soil. Small seeded species are more likely to survive ingestion compared with large seeded species (Carter, 1980). The seeds of these four clover species were less than 2 mg (Table 7.4). About 23% of 2 mg seed (Russi *et al.*, 1992a) and 45% of 1mg (Edward *et al.*, 1998) were reported to pass the digestive tract unharmed.

7.4.2 Burried seeds versus seeds on soil surface

The population of buried seeds was dynamic. It decreased rapidly during the first three months after burial and then slowly there after (Figure 7.7). Over the 18-month burial duration, ‘Cefalu’ arrowleaf had the most viable seeds recovered in the soil. The longevity of seeds in the soil is believed to be caused by their impermeable seed coat (Barton, 1961). Impermeable or hard seed coat prevents water and oxygen from entering into the seed, thus prevents germination. In this study, the germination of seeds buried at 170 mm depth contributed < 6% (Figure 7.6) to the depletion of seed populations. The disappearance of seeds was mainly due to seed mortality from seed decay and accounted for about half of the buried seed population.

Longer burial duration was found to decrease the chances of seed germination (Figure 7.6). The reason for declining seed germination with burial time could be due to poor soil aeration resulting from compaction (Barton, 1962; Lewis, 1961). The germination of buried seeds was much lower (< 6%) than germination of seeds on the soil surface (11 – 25%) (Figure 7.8). This was consistent with James and Rahman (2003), Loi *et al.*, (1999) and Pe (1978) who reported that seed longevity and seed softening in the soil varied with depth of burial. Low germination at deeper burial may be due to low oxygen supply (Harris, 1959), high carbon dioxide levels (Wesson and Wareing, 1969) and low and small fluctuating temperatures (Pe, 1978). In contrast, temperatures on the soil surface were much higher and had greater fluctuation (Figure 7.2a) than those below the surface (Figure 7.2b,c). Therefore, higher germination of seeds on the soil surface is believed to be attributed to more variable soil temperature fluctuation and greater aeration. High summer temperatures and wide temperature fluctuation between 15 and 60 ^{o}C has been reported to accelerate the breakdown of hardseeds of annual legumes (Quinlivan, 1961; Quinlivan, 1971; Taylor, 1981; Taylor and Ewing, 1992). However, these reports were based on studies conducted in Australia climate where summer temperatures are extreme. This may not be applicable to New Zealand climate because the maximum average summer temperatures at soil surface do not reach 60 ^{o}C (Figure 7.2).

In this study, the effect of summer temperature was unable to be determined precisely because seeds were initially buried and sown in April (mid autumn) (Section 7.2.4) and most germination occurred during the three months following burial (Figure 7.6,Figure 7.7). This suggests that cold fluctuating temperatures also influenced the breakdown of hard seeds. Since germination results in depletion of seed populations, high germination on the soil surface implies that seeds that lay on the ground did not retain their viability for any longer than buried seeds. On arable land, the majority of seeds that dropped on the ground will be buried at various depths during land preparation. Thus, seeds that are buried deep in the soil survive longer and are able to maintain the seed population for many years. This is unlikely to be the case in pastures which are expected to last for 5 to 10 years in New Zealand conditions.

7.4.3 Breakdown of hard seeds

The seeds of annual clovers germinated at the species' optimum temperature (constant temperature) (Section 7.2.3) showed an increment in the rate of hardseed breakdown across the 18-month period following seed harvest (Figure 7.5). The only exception was 'Mihi' Persian clover, which had soft seeds ~ 95% germination throughout the experiment period. In 'Cefalu' arrowleaf clover, the rate of hardseed breakdown differed with sowing dates (Figure 7.5a). Seeds harvested from SD1 (26 Feb10) were the hardest and therefore had the lowest germination compared with seeds from other sowing dates. Previous reports indicate a longer maturation period increases the thickening of seed coat and enhances the resistance towards seed softening (Aitken, 1939; Quinlivan, 1965). However, the duration of seed filling (Table 4.9), ambient temperatures during seed production (Figure 4.9a) and individual seed weight (Table 7.4) were unable to explain the variation in hardseededness among the sowing dates. Thus, the factor contributing to this discrepancy is still unclear.

Except for 'Mihi' Persian clover, the germination of seeds harvested from SD2 (24 Mar 10) that was conducted at a constant optimum temperature (Figure 7.5) reflected the seedling emergence that occurred in the field (Figure 7.8) which also used the same seeds. This showed that seed softening in the laboratory was comparable to that in the field (Table 7.8) despite the fact that seeds experienced different temperature conditions in the incubator (constant optimum temperature) and in the soil (fluctuating temperatures).

Table 7.8 Laboratory germination (%) and field emergence (%) of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers on 12 April 2011 at Lincoln University, Canterbury, New Zealand.

Species	Germination (%)	Field emergence (%)
'Cefalu' arrowleaf	24.5	11.3
'Bolta' balansa	29.0	25.5
'Prima' gland	13.0	15.7
'Mihi' Persian	97.0	19.2

All species used the same seeds harvested from SD2 (24 Mar 10).

According to Taylor (1981), constant temperatures alone produced few soft seeds. In natural conditions, seeds on the soil surface are exposed to a large amplitude of fluctuating temperatures which induces the breakdown of hardseededness (Quinlivan, 1961, 1966; Quinlivan, 1971). Therefore, many seed softening studies under controlled conditions use fluctuating temperatures to simulate the natural conditions in the field (Norman *et al.*, 2006; Quinlivan, 1961, 1966; Taylor, 1981; Taylor, 1993). In studies of hardseed breakdown of subterranean clover, a fluctuating temperature of 60/15 ^{o}C has been shown to accelerate seed softening by disrupting the strophiolar region and increases the strophiolar permeability to water (Norman *et al.*, 2006; Taylor, 1981; Taylor and Ewing, 1992). It is unknown how fluctuating temperatures will affect the seed softening of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers since no fluctuation temperature treatment was applied in this present study. Future work on hardseed breakdown should focus on the effect of fluctuating temperatures, scarification and pre-conditioning treatments.

7.5 Conclusions

1. The seed yield ranged from 221 to 914 kg/ha for ‘Cefalu’ arrowleaf, 859 to 2309 kg/ha for ‘Bolta’ balansa, 536 to 2370 kg/ha for ‘Prima’ gland and an average of 814 kg/ha for ‘Mihi’ Persian clover.

2. In the first year of regeneration, ‘Bolta’ balansa and ‘Prima’ gland clovers were the earliest emerged species. Based on the germination experiment, approximately 13% of ‘Bolta’ balansa and 3% of ‘Prima’ gland clovers seed bank emerged following rainfall in January 2011. About 3% of ‘Cefalu’ arrowleaf and 96% of ‘Mihi’ Persian clovers seed population emerged in March 2011. ‘Bolta’ balansa had the highest regeneration cover of 91%, followed by ‘Prima’ gland (65%), ‘Mihi’ Persian (17%) and ‘Cefalu’ arrowleaf clovers (6%).

3. ‘Mihi’ Persian clover seeds were 95% soft. After 22 months from seed harvest, 19-56% hardseeds of ‘Cefalu’ arrowleaf and 12% of ‘Prima’ gland clover seeds were softened at their optimum temperature. ‘Bolta’ balansa clover had 30-36% breakdown of hardseeds after 17 months.

4. Germination and survival of buried seeds in the soil decreased over the 18 month period. The percentage of emerged seedlings on the top of the tubes placed in the field was 24.9% for ‘Bolta’ balansa, 19.2% for ‘Mihi’ Persian, 15.1% for ‘Prima’ gland and 10.9% for ‘Cefalu’ arrowleaf clover over a period of 12 months.

5. ‘Mihi’ Persian clover did not persist because it did not produce any hard seed. For ‘Cefalu’ arrowleaf, ‘Bolta’ balansa and ‘Prima’ gland clovers, the recovery of hard seeds in the soil after 18 months showed that there was adequate seed production to sustain an on-going regeneration in subsequent years.

8 General discussion

8.1 Introduction

Previous chapters have defined the development (Chapters 3-5), growth (Chapter 6) and persistence (Chapter 7) of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers in controlled and dryland pasture conditions. This general discussion aims to suggest how this information may be transferred into New Zealand farm system with focus on hill and high country pastures. Management for successful inclusion of these annual clovers are discussed and topics which require further research are indicated.

8.2 Establishment of annual clovers

Cardinal temperatures for germination of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers showed that these species are adapted to a broader range of temperatures compared with haresfoot, suckling, cluster, striated (known as adventive clovers), subterranean and white clovers (Table 8.1).

Table 8.1 Cardinal temperatures for germination of 11 clover species.

	Cardinal temperatures (^oC)		
Clover species	T_b	T_{opt}	T_{max}
‘Cefalu’ arrowleaf	1.4	19.7	34.8
‘Bolta’ balansa	2.6	19.7	33.9
‘Prima’ gland	-0.1	16.2	34.1
‘Mihi’ Persian	4.5	32.6	45.4
Haresfoot[1]	0.4	11.8	25.6
Suckling[1]	0.3	13.8	24.4
Cluster[1]	-1.1	12.9	27.0
Striated[1]	1.4	11.6	27.7
‘Woogellenup’ subterranean[2]	0	20-25	35.0
‘Rosabrook’ subterranean[2]	-0.1	19.2	29.1
‘Demand’ white clover[3]	3.5	27.1	38.6

Note: [1]Lonati *et al*. (2009), [2]H.Nori (unpublished data), [3]Black *et al*. (2006).

This means that arrowleaf, balansa, gland and Persian clovers can inhabit the same ecological niche with the adventive, subterranean and white clovers, and survive beyond temperatures where some other species cannot. Specifically, Persian clover has greater tolerance to high temperatures where it has the fastest germination rate at 33 °C and 40% of its seed population germinated at 40 °C. During summer, near soil surface temperature has been reported to exceed 30 °C in the Bay of Plenty (Watson *et al.*, 1996) and >40 °C in Ashley Dene dryland farm, Canterbury (R.Sim, unpublished data). The combination of these extreme temperatures and drought caused stolon death and disappearance of white clover populations in dryland region during summer in each year. This situation may be an advantage for Persian clover to replace white clover because it could flourish at extreme high temperature. Furthermore, it has a longer lifespan than subterranean clover which could extend the grazing duration.

Table 8.2 The thermal time requirements for germination, emergence, first (spade) leaf, axillary leaf initiation and phyllochron for 10 clover species.

	Thermal time requirement (°C d) above $T_b = 0$°C				
Clover species	Germination	Emergence	1st leaf	Axillary leaf	Phyllochron
‘Cefalu’ arrowleaf	34	90	196	453	60
‘Bolta’ balansa	32	90[a], 75[b]	169	326	45
‘Prima’ gland	28	96	175	304	34
‘Mihi’ Persian	25	87[a], 55[c]	154	439	65
Haresfoot[1]	20	n.a.	141	351	53
Suckling[1]	23	n.a.	146	341	62
Cluster[1]	28	n.a.	165	447	60
Striated[1]	26	n.a.	160	355	52
‘Mt. Barker’ subterranean[1]	26	112[2]	160	437	55
‘Demand’ white clover[3]	40	109	208	532	94

Note: [1]Lonati *et al.* (2009), [2]Moot *et al.* (2000), [3]Black *et al.* (2006), [a]5.1-10.6 °C, [b]12.1-20.7 °C, [c]12.1-23.9 °C, n.a., data not available. To enable direct comparison among species, time to axillary leaf and phyllochron were based on 16 hour photoperiod.

The establishment of all annual clover species was much faster than perennial white clover with less thermal time required to complete each development stage (Table 8.2). This means that rapid establishment of annual clovers have an advantage to compete vigorously for light because they reached exponential leaf production quicker than white clover. As a consequence, annual clovers have been shown to produce more above ground dry matter than the perennial white clover (Moot *et al.*, 2000; Murray, 2012). In a glasshouse study, Murray (2012) showed that ‘Bolta’ balansa and ‘Prima’ gland clovers produced heavier shoot weights of 0.14 g/plant and 0.05 g/plant respectively, in contrast to 'Nomad' white clover (0.03 g/plant) after 75 days from sowing. White clover has slow winter growth and it stops growing when temperature is less than 8 ^{o}C. By the time temperature starts to increase in September, the compensation of white clover growth is too late for early lambing ewes in many dryland areas because it does not provide sufficient feed. In addition, the peak growth of white clover in late-spring and summer in dry east coast region is often curtailed by drought. This problem can be alleviated by the inclusion of annual clovers in dryland pasture. These annual clovers grew rapidly in winter and produced high biomass in early spring (Table 8.3) which could provide high quality feed for grazing during lambing in August and September. In this study, monocultures of late-February sown crops yielded 1452 kg DM/ha for arrowleaf, 4008 kg DM/ha for balansa, 1948 kg DM/ha for gland and 3015 kg DM/ha for Persian clover by mid-September. For late March sown crops, its early spring production ranged from 445 kg DM/ha in arrowleaf clover to 2058 kg DM/ha in balansa clover.

Table 8.3 Legume dry matter (DM) yield (kg/ha) and winter growth date (kg DM/ha/day) for 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on 26 February and 24 March 2010 at Iversen 9 field, Lincoln University, New Zealand.

Species	Sowing date	DM yield (kg/ha)		Winter growth rate
	(2010)	4 Jun	14 Sep	(kg DM/ha/day)
'Cefalu' arrowleaf	26 Feb	746	1452	7
	24 Mar	-	445	-
'Bolta' balansa	26 Feb	972	4008	30
	24 Mar	-	2058	-
'Prima' gland	26 Feb	407	1948	15
	24 Mar	-	1186	-
'Mihi' Persian	26 Feb	1257	3015	17
	24 Mar	-	1051	-

Seedling growth rate and consequently its dry matter production are also dependent on the weight of seed. Heavier seeds produce a larger leaf area and consequently heavier seedlings (Black, 1958). This is because heavier seeds contain a larger embryo (food reserves) and produce larger cotyledons that capture more incoming solar radiation. Similarly, although balansa (1.1 mg/seed) and gland (1.0 mg/seed) clovers established quicker than subterranean clover (6.7 mg/seed) (Table 8.2), they are often outyielded by subterranean clover in a mixed sward (Dear *et al.*, 2002; Monks, 2009) because they have small seedlings which are less competitive. In a mixed sward, small seedlings are shaded and this decreases their ability to compete for light. Therefore, it is recommended that balansa and gland clovers should be sown in a monoculture or with slow establishing species such as cocksfoot (Monks, 2009) rather than in a mix with species like perennial ryegrass.

When making decisions on suitable sowing time and rate and selecting companion species, one should consider both development and growth rate (Moot *et al.*, 2000). Slow establishing species such as white clover should be sown in spring when temperature increases and it can be sown together with other slow species like cocksfoot and timothy. For autumn sowing, fast developing species such as annual clovers can establish before the onset of cooler temperatures. When sowing mixtures of species with different seedling growth rates (owing to their seed size), it is suggested that heavier seeded species should be sown at low rates (Cullen, 1958). This allows a large population of plants from the small seeded species to increase their leaf area index (Shibles and Weber, 1966) and compete with large seeded species. To support this justification, Evans *et al*. (2002) reported that higher winter production of 'Bolta' balansa and 'Nitro' Persian clovers compared with 'Leura' subterranean clover were attributed to their higher plant density. Another option to improve competitiveness of small seeded species in a mixed sward is to introduce grazing after emergence (Dear *et al.*, 2002) to prevent larger-seeded species such as subterranean clover from shading the small seedlings.

The time of axillary leaf production can be used as an indicator of the time for commencement of first grazing (Moot *et al.*, 2003a), provided that the seedlings pass the 'pull test' for grazing. In this experiment, seedlings of arrowleaf, balansa, gland and Persian clovers passed the pull test at the axillary leaf stage. The time of axillary leaf appearance was driven by temperature and modified by photoperiod at emergence (Figure 3.14). Based on long term mean temperature data and calibration model for thermal time to axillary leaf production (Table 3.11), theoretical chronological time to first grazing of arrowleaf, balansa, gland and Persian clovers can be estimated for a range of sites in New Zealand, assuming that soil moisture is non-limiting (Table 8.4).

Table 8.4 Chronological time (days) to first grazing of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers at four locations in New Zealand with four sowing dates (days calculated from NIWA meteorological data, using a T_b of 0 ^oC).

Species	Location	Sowing date			
		1 Feb	1 Mar	1 Apr	1 May
'Cefalu' arrowleaf	Lincoln	39	59	69	90
	Blenheim	36	53	61	65
	Lake Tekapo	42	66	83	133
	Alexandra	38	59	75	120
'Bolta' balansa	Lincoln	26	37	56	76
	Blenheim	24	33	50	65
	Lake Tekapo	28	41	68	112
	Alexandra	25	37	61	102
'Prima' gland	Lincoln	28	35	50	69
	Blenheim	26	32	44	58
	Lake Tekapo	30	39	60	101
	Alexandra	27	35	54	92
'Mihi' Persian	Lincoln	31	42	62	82
	Blenheim	29	38	54	69
	Lake Tekapo	34	47	74	120
	Alexandra	30	42	67	109

8.3 Flowering and growth of annual clovers

Time of flowering in annual clovers was dependent on sowing date or the date of seeds dropped on the ground after year 1. Late February sown crops that emerged in early March into a decreasing 13.6 hour photoperiod took the longest time to produce their first flower (Figure 4.4). This resulted in the largest amount of vegetative growth prior to flowering and therefore produced the highest total dry matter (Figure 6.2). For example, balansa clover sown in late February required 1730 ^oC d from emergence to flower in mid-September (Figure 4.4b). At this time, it had produced 4.4 t/ha of dry matter and continued to accumulate beyond 17.5 t/ha as it reached physiological maturity in mid-November (Figure 6.2b). In contrast, July sown balansa clover that emerged into an increasing 10.5 hours photoperiod only required 870 ^oC d to flower on 22nd October. This was half the thermal time of late February sown crops. Because of their short vegetative growth, July sown balansa clover only produced 0.6 t/ha biomass at first flower and accumulated up to 3.9 t/ha biomass at physiological maturity in early December. Annual clovers that emerged into an increasing photoperiod towards spring rapidly become reproductive and mature thus limit their biomass productivity. In contrast, annual clovers that emerged in autumn into shortening days and falling temperatures delay their flowering by producing more leaves (Figure 4.3). They will flower when temperature and photoperiod increases in spring. Therefore, it is recommended to sow annual clovers in autumn, preferably early to mid-March to maximise dry matter production for winter grazing (Table 8.3) and to enable crops to set seed before summer drought. It should be noted that autumn sown crops have a longer duration of lag phase (Section 6.3.1.2), thus making them vulnerable to winter annual weed invasion. Therefore, weed control is important for management of autumn sown crops. The influence of increasing photoperiod in acceleration of flowering time showed that annual clovers can be sown later in spring and still produce seed but their productivity will be limited by summer drought (Table 8.5).

Safe grazing can commence following the production of the first axillary leaf. For arrowleaf clover, this was leaf six but in the other three species, it was leaf five. Table 8.5 showed that crops that emerged earlier in March (late February sown) reached exponential leaf production in April –May which indicates their ability to provide grazing in winter for pregnant stock. Later flowering arrowleaf and Persian clovers can extend grazing into September and November, respectively. This is because these species had a longer growth duration and matured in December. They are suitable to be grown in areas of high spring rainfall such as coastal southern Marlborough or the east coast of the North Island. A field trial conducted at Clarence Bridge, south Marlborough in 2011 showed that arrowleaf and Persian clovers sown on 26th March produced 15.1 and 19.5 t/ha of dry matter in December (Lucas, 2012) which was higher than the production in Iversen 9 field, Lincoln University, Canterbury (Figure 6.2a,d) which commonly experiences severe summer drought (Figure 3.1). In addition, tolerance of Persian clover to high temperatures indicates it can be grown successfully in warmer climates. Arrowleaf clover does not tolerate wet soils in contrast to Persian clover. Personal observation on arrowleaf and Persian clovers grown on the Wakanui silt loam soil in Iversen 9 field, Lincoln University found that during a wet winter in 2010, significant areas in the field were flooded for 1-2 days which resulted in chlorosis among arrowleaf clovers while Persian clovers were unaffected. Arrowleaf clover had slower seedling growth in winter compared with the other three species. Its linear growth phase only began in mid spring.

Slow seedling growth of arrowleaf clover caused it to be susceptible to weed invasion (Table 6.2). Therefore, weed control during winter is necessary to reduce competition for light and moisture. This can be done by herbicide application.

Table 8.5 Dates of each phenological stage of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown on three dates in 2010 at Iversen 9 field, Lincoln University, New Zealand.

		Sowing date (2010)		
Species	Phenology	26-Feb	7-Jul	25-Sep
'Cefalu' arrowleaf	Emergence	9-Mar	23-Jul	5-Oct
	Axillary leaf	8-May	7-Oct	8-Nov
	Flowering	21-Sep	8-Nov	16-Dec
	Physiological maturity	18-Dec	11-Jan	2-Feb
'Bolta' balansa	Emergence	10-Mar	21-Jul	4-Oct
	Axillary leaf	4-Apr	9-Sep	2-Nov
	Flowering	14-Sep	22-Oct	6-Dec
	Physiological maturity	18-Nov	4-Dec	31-Dec
'Prima' gland	Emergence	12-Mar	22-Jul	5-Oct
	Axillary leaf	11-Apr	8-Sep	1-Nov
	Flowering	16-Jul	12-Oct	20-Nov
	Physiological maturity	21-Oct	9-Dec	30-Dec
'Mihi' Persian	Emergence	8-Mar	22-Jul	3-Oct
	Axillary leaf	5-Apr	24-Sep	3-Nov
	Flowering	16-Nov	23-Nov	16-Dec
	Physiological maturity	28-Dec	5-Jan	18-Jan

Gland clover was the earliest flowering species which makes it suitable to areas which dry out in the end of October/early November. It was established productively at Ashley Dene dryland farm, Canterbury (Murray, 2012) and Marlborough (D. Avery, pers. com). However, its short lifespan due to early maturity limit its dry matter productivity thus makes gland clover the least productive of these four species. Figure 6.10 shows that canopy growth did not accelerate until spring when temperature began to rise. As a result, about 70% of total dry matter production occurred during spring (Figure 6.2). Therefore, gland clover matured as early as 21st October (Table 8.5) and did not allow it to utilise the whole spring season to maximize its growth. Mid-flowering balansa clover was the most prolific species which had the highest radiation use efficiency (Table 6.12) to produce high dry matter (up to 17.5 t/ha) within a short growing season (Figure 6.2b). Balansa clover was also shown to tolerate water-logging during winter 2010 in Iversen field which indicates its suitability to grow in areas of wet winter. Differences in flowering time among these annual clovers require different grazing management for individual species. Grazing should be spelled in mid-July for gland, mid-September for both arrowleaf and balansa clovers and mid-November for Persian clover to allow them to set seed (Table 8.5). Following physiological maturity, grazing can resume from late October for gland, 3rd week of November for balansa, mid-December for arrowleaf and January for Persian clover. The species will then survive summer drought as seeds and germinate following autumn rain to produce a new generation of seedlings.

8.4 Seed production and re-establishment of annual clovers

The future of annual clovers in a pasture system relies on high production of hard seeds to survive (Boswell *et al.*, 2003; Jansen and Ison, 1996; Kendall and Stringer, 1985) (Section 2.9.1). With a sowing rate of 4 kg/ha, balansa (1.1 mg/seed) and gland (1.0 mg/seed) clovers produced up to 2300 and 2370 kg/ha of hard seeds, respectively (Table 7.2). Heavier seeds of arrowleaf (1.2 mg/seed) and Persian (1.6 mg) clovers were sown at 6 and 5 kg/ha respectively and yielded up to 680 and 850 kg/ha of hard seeds. This shows that annual clover species have different reproductive strategies to persist within an ecological niche. Small seeded species such as balansa and gland clovers produced large number of seeds to secure a high population of small seedlings. In contrast, arrowleaf and Persian clovers with larger seed size are dependent on a few populations of large seedlings to achieve large ground cover.

The survival of a species depends on a large production of hard seeds and the ability of these seeds to germinate and produce reasonable seedling populations in the following season (Section 2.9.2). In the second year (July, 2011), 23% of balansa, 19% of Persian, 14% of gland and 10% of arrowleaf clover seedlings emerged in the field (Figure 7.8). These populations of seedlings recruitment grew to produce a maximum ground cover of 91% in balansa, 17% in Persian, 65% in gland and 6% in arrowleaf clover over the next three months (Figure 7.4). As a result, balansa, Persian and gland clovers produced high dry matter in the 2nd year (Figure 6.2) but arrowleaf clover did not. This indicates that the proportion of balansa, Persian and gland clovers' seeds that soften in the 2nd year was sufficient to regenerate a similar level of dry matter in the second year. For arrowleaf clover, the high percentage of hardseededness (~97%) is the main cause of the low population of seedling emergence and consequently low biomass production in the 2nd year. This suggests the use of arrowleaf and Persian clovers as a specialist pasture crop rather than an on-going regeneration crop.

Following seed maturity, the ideal time for seed softening is during summer because daily temperature fluctuation on the soil surface is wide (30/15 °C) thus inducing rapid breakdown of seed coat impermeability. However, the timing of hardseed breakdown is also dependent on how soon the seeds are shed from the parent plant. It appears that these four species of annual clover have different mechanism of seed dispersal. Balansa clover has the quickest and easiest way of dropping seeds because its pods burst at harvest maturity (Table 4.2). This guaranteed all seeds were laid on the ground without any aid of a dispersal agent. For arrowleaf and gland clovers, wind or animal grazing and trampling were needed to disperse their seeds, otherwise they would require a longer time for the seeds to drop naturally to the ground. In this case, early maturity of gland clover may give ample time for the the seeds to drop on the ground by summer but this is not the case for late maturing arrowleaf clover. For Persian clover, the pods burst at maturity, but seeds are trapped inside the inflated calyx and did not drop to the ground (Plate 22). Therefore, Persian clover would require animal grazing to spread its seeds via faeces. As these plots were not grazed until 29th April 2011 (Section 7.2.2), this action was too late to facilitate seed softening of arrowleaf clover and seedling establishment of Persian clover. The failure in re-establishment of these two species could have been avoided if grazing was introduced much earlier in summer.

In April 2012, there was still 204 kg/ha of arrowleaf, 374 kg/ha of balansa and 398 kg/ha of gland clover hard seeds in the soil (Figure 7.7). These are substantial populations of hard seeds relative to the amount of seed sown. This could then be managed for regeneration in subsequent years. In October 2012, a large proportion of balansa and small proportion of gland clovers were observed in the same plots at Iversen 9, Lincoln University. No arrowleaf or Persian clovers were found in 2012. In April 2013, there was a significant proportion of balansa clover seedlings found but none from the other three species. The ability of balansa clover to keep an on-going field population agrees with Monks (2009) who concluded that this species produced a population of seeds that could be relied on for up to four years. In contrast, ‘Mihi’ Persian clover is a soft seeded cultivar, thus there were no seeds remaining in the soil after three months burial (Figure 7.7). The seeds were lost either through germination or decayed.

Given that these four species of annual clover have different characteristics of hardseededness and ability to re-establish in the following season, each species would require different management for successful establishment on dryland farms. Among these four species, balansa is the most reliable species which may only require seed set every four years to maintain the seed bank. Gland clover may require sowing after two years while arrowleaf clover with high levels of hardseededness would depend on re-sowing in the 2nd year. For Persian clover to persist in the pastoral system, a switch to a hardseeded cultivar such as ‘Nitro’ is recommended. Australian research recommends cultivars of annual species with high levels of hardseededness for suitable use in ley farming and for longer survival in the soil (Loi *et al.*, 2005). This is because the environmental conditions in Australia are much warmer which induces rapid seed loss from the soil through seed softening. In contrast, New Zealand does not experience the extreme temperature conditions, thus the rate of seed loss would be expected to be slower. This also means that rate of hardseed breakdown would be slower for re-establishment in the following season. In this case, New Zealand may be interested in cultivars with moderate levels of hardseededness.

8.5 Oversowing annual clovers in hill and high country

Oversowing field trials in Castle Hill, Canterbury (700 m a.s.l.), Lees Valley, Canterbury (434 m a.s.l.) and Breach Oak, Blenheim (193 m a.s.l.) in 2011 showed promising yields of ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers (Table 8.6) for inclusion in hill and high country farms.

Table 8.6 Dry matter at full flower for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown in autumn 2011 at Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim, New Zealand.

	Dry matter (t/ha) ± S.E. at full flower from three locations		
Species	Castle Hill	Lees Valley	Breach Oak
‘Cefalu’ arrowleaf	9.8 ± 7.99	8.7 ± 1.53	12.8 ± 5.35
‘Bolta’ balansa	9.5 ± 2.56	7.1 ± 0.63	5.3[b] ± 3.02
‘Prima’ gland	5.6 ± 0.18	2.3 ± 0.42	4.8[b] ± 2.09
‘Mihi’ Persian	12.5[a] ± 0.77	6.2[a] ± 0.70	8.9 ± 3.85

[a]first flower, [b]physiological maturity. S.E., standard error. Crops were sown on 11 March at Castle Hill, 29 March at Lees Valley and 15 April at Breach Oak.

Interestingly, these yields were higher than those grown on flat land in Lincoln University, Canterbury in 2010 (Table 6.3). This indicates that arrowleaf, balansa, gland and Persian clovers grew successfully on areas ranging from dry warm to dry cool, high altitudes.

The thermal time requirements for vegetative phenology of commercial annual clovers (arrowleaf, balansa, gland and Persian clovers) were similar to the adventive annual clovers (Table 8.2). They all took the same time to reach axillary leaf appearance, which is between 304 - 453 ^{o}C d from sowing. Because of their similarity in temperature response (Table 8.1) and establishment rate (Table 8.2), this suggests that arrowleaf, balansa, gland and Persian clovers may be suitable to be oversown in hill and high country where the adventive clovers are abundantly present. The specific habitat of adventive annual clover species and their flowering characteristics may indicate suitable areas where arrowleaf, balansa, gland and Persian clovers could fit. For example, gland clover which flowers and matures early can be sown in dry areas with low rainfall at lower altitude on the north faces where haresfoot clover is abundantly present. Mid flowering balansa clover which has intermediate maturity is the most flexible species and can be grown on wide range of environment. Personal observation in Iversen field, Lincoln University and Lees Valley, Canterbury suggests that this species is easily widespread by grazing livestock. The ability of balansa clover to tolerate water logging indicates this species may have a place in significant areas in the North Island hill country where mudstone soil saturate in winter and dry out quckly in summer (Moot, 2012). Balansa clover has similar characteristics with suckling clover which therefore suggests a similar wide range of micro-habitats where balansa clover can be sown. In addition, the ability of balansa clover to grow well in cooler environment (Figure 6.8) suggests this species may be able to grow at higher altitudes. The presence of cluster and suckling clover in areas of intermediate to high rainfall indicate the potential area where late flowering arrowleaf and Persian clovers may fit. Because arrowleaf and Persian clovers have a long growth duration and therefore mature late, these species are most suited on the south facing slopes where soil moisture deficit occurs in late summer.

Success in oversowing these four commercial annual clovers on hill and high country farm is dependant on the correct time of sowing. In winter, pasture growth is hampered by extreme low temperature and seedlings can be killed by frost. As temperature increases in spring, pasture growth accelerates but then rapid soil moisture deficit in late spring limit its further growth. Therefore, it is important to select a suitable sowing time to avoid seedlings being killed by winter frost while at the same time the crop must be able to set seed and complete its life cycle before the onset of drought. When crops were sown in autumn (11th and 29th March in Castle Hill and Lees Valley, and 15th April in Breach Oak), leaf production of annual clovers was slow due to falling temperatures and declining photoperiods (Figure 5.13). As a consequence, plants on 1st June only produce 3-4 leaves on the main stem for 'Cefalu' arrowleaf, 4-5 leaves for 'Bolta' balansa, 4-6 leaves for 'Prima' gland and 3-5 leaves for 'Mihi' Persian clover (Table 8.7).

Table 8.7 Number of leaves on the main stem of 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown in autumn 2011 at Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim, New Zealand.

	1st June			1st September		
Species	Castle Hill	Lees Valley	Breach Oak	Castle Hill	Lees Valley	Breach Oak
Arrowleaf	4	4	3	7	8	7
Balansa	4	5	4	8	10	9
Gland	6	5	4	8	10	10
Persian	5	3	4	7	8	8

Crops were sown on 11th March at Castle Hill, 29th March at Lees Valley and 15th April at Breach Oak.

This limits the productivity of autumn sowing in high country as winter growth is insufficient (due to extremely low temperature) for grazing. Thus stock will not graze until canopy growth accelerates in spring. Another concern about oversowing in March is that germination and consequently seedling emergence will totally depend on the arrival of rain which is uncertain until May. But oversowing in May would risk emerged seedlings being lifted by frost heave. Power (2007) reported that thermal time accumulation of 1355 ^{o}C d from July to December is sufficient for adventives annual clovers at an altitude of 450 m a.s.l. in Mt Grand to complete their life cycle and therefore suggested spring sowing for annual clovers. However, the researcher did not quantify thermal time requirements from emergence to physiological maturity for these adventives species and they may have already emerged during autumn.

Table 8.8 Thermal time accumulation (°C d) from sowing to physiological maturity and estimated date for 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers to emerge, flower and mature for proposed sowing date on 1st September in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim, New Zealand.

Location	Species	Tt accumulation (°C d)	Sowing date: 1st September Emergence	1st Flower	Phys. maturity
Castle Hill, Canterbury	Arrowleaf	1851	15-Sep	27-Dec	26-Feb
	Balansa	1330	15-Sep	10-Dec	17-Jan
	Gland	1270	16-Sep	29-Nov	15-Jan
	Persian	1795	15-Sep	3-Jan	17-Feb
Lees Valley, Canterbury	Arrowleaf	1859	13-Sep	20-Dec	17-Feb
	Balansa	1346	13-Sep	3-Dec	9-Jan
	Gland	1295	14-Sep	23-Nov	10-Jan
	Persian	1852	13-Sep	30-Dec	6-Feb
Breach Oak, Blenheim	Arrowleaf	1870	10-Sep	4-Dec	28-Jan
	Balansa	1368	10-Sep	21-Nov	25-Dec
	Gland	1310	10-Sep	10-Nov	24-Dec
	Persian	1898	9-Sep	15-Dec	21-Jan

Note: Tt accumulated from sowing to physiological (Phys.) maturity.

Table 8.8 calculates the thermal time accumulation from sowing to physiological maturity and predicted date of emergence, flowering and maturity for four commercial annual clover species if they were to be sown later in spring (1st September) at three locations in the hill and high country. Thermal time accumulation to complete the life cycle of annual clovers were slightly different among locations because species emerge at different time (earliest: Breach Oak, latest: Castle Hill) due to differences in mean soil temperature (Castle Hill: 6.0 °C, Lees Valley: 6.9 °C, Breach Oak: 9.2 °C). Differences in time of seedling emergence will result in differences in flowering time due to photoperiod modification (Figure 4.4) and therefore affect total thermal time accumulation. Spring sowing of arrowleaf and Persian clovers may be too late for them to complete their vegetative growth into December as soil moisture deficits could limit their productivity. In contrast, balansa and gland clovers which mature in late December – mid January may be the potential species for spring sowing. When sowing annual species in spring, one should consider the trade-off between development and growth. As development gets faster, growth is reduced because of the short growth duration. On the contrary, sowing in early March resulted in all species flowering before 7th December (Table 8.9) and crops produced high dry matter yields (Table 8.6).

Prior to oversowing on hill and high country, the area to be sown should be grazed to remove resident vegetation and increase the chances of broadcasted seeds to fall on the soil surface. Seeds of annual clovers should be coated and inoculated before oversowing on uncultivated land to increase rhizobia survival (Lambert *et al.*, 1985). Autumn sowing before May is recommended so that seedlings could establish before the onset of freezing temperature, to produce high dry matter and to allow crops to mature before summer drought. Immediately after oversowing, the area should be heavily set-stocked for 1-2 days to trample seed into the soil which could increase the chances of seed germination (Lambert *et al.*, 1985). Safe grazing of annual clovers can commence following time of axillary leaf production and grazing should be spelled before the appearance of first flower. Based on time of flowering of autumn sown crops in Castle Hill, Lees Valley and Breach Oak, animals should be removed from the paddock before the following dates (Table 8.9). Following physiological maturity (Table 8.10), the animals should be re-introduced into the paddock for intensive grazing to open the sward and assist in seedling establishment in the second year. Little is known about the ability of seedlings to re-generate the following autumn under hill and high country conditions. On 6 April 2013, Lucas (pers. com) observed numerous seedlings of balansa, subterranean and adventives clovers re-generated at the spade leaf stage following 27 mm rainfall over the last three weeks at 500 m a.s.l at Mt. Grand, Central Otago. This indicates the potential for the introduction of annual clovers into the high country.

Table 8.9 Date of flowering for Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim, New Zealand.

	Date of flowering		
Species	Castle Hill	Lees Valley	Breach Oak
'Cefalu' arrowleaf	5-Nov	24-Oct	18-Oct
'Bolta' balansa	19-Oct	9-Oct	29-Sep
'Prima' gland	8-Oct	27-Sep	15-Sep
'Mihi' Persian	6-Dec	26-Nov	25-Nov

Crops were sown on 11th March at Castle Hill, 29th March at Lees Valley and 15th April at Breach Oak.

Table 8.10 Estimated date of physiological maturity for Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clovers sown in Castle Hill, Canterbury; Lees Valley, Canterbury and Breach Oak, Blenheim, New Zealand.

	Date of physiological maturity		
Species	Castle Hill	Lees Valley	Breach Oak
'Cefalu' arrowleaf	22-Jan	22-Jan	15-Jan
'Bolta' balansa	19-Dec	13-Dec	24-Nov
'Prima' gland	23-Dec	22-Dec	16-Nov
'Mihi' Persian	22-Jan	22-Jan	20-Jan

Crops were sown on 11th March at Castle Hill, 29th March at Lees Valley and 15th April at Breach Oak.

8.6 Future work

8.6.1 Nodulation and nitrogen fixation

Future work needs to assess the effect of resident rhizobia in the soil on nodulation and how much nitrogen is fixed by these annual clovers. There is also a need to identify specific rhizobia strains for successful inoculation of individual species.

8.6.2 Grazing management

Little is known on grazing top flowering annual clovers in New Zealand. Specifically, grazing frequency and intensity relative to life cycle of each species, animal performance, nutritive composition, how fast the species recover following grazing and dry matter production at grazing interval needs further study for grazing management.

8.6.3 On-going seedling regeneration

Observation of seedling regeneration for at least three years would be required to determine the seedling recruitment pattern and seed bank dynamics. The outcome would then suggest management options to keep an on-going population of these species in the field, i.e. whether there is a need to re-sow or allow seed set every second or third year. Futher work on summer grazing management in relation to seed softening and the effect of fluctuating soil surface temperatures are necessary. Recovery of seeds from ingestion by grazing animals and their viability require investigation.

8.7 Conclusions

The research presented in this thesis has quantified development and growth of arrowleaf, balansa, gland and Persian clovers under controlled environment and field conditions. These results were discussed in relation to dryland farm management. Specific conclusions were:

1. Phenology development from germination to the appearance of first leaf were predominantly driven by temperature. These were quantified using soil temperature on the basis that location of meristem apex is still below ground. Following stem extension, development was quantified using air temperature.

2. Leaf appearance and time to flowering were driven by temperature and modified by photoperiod at emergence. Crops that emerged into a decreasing photoperiod had longer vegetative growth and flowered much later compared with those that emerged into an increasing photoperiod. Therefore, autumn sowing for annual clovers is recommended to maximise dry matter production and to complete crop life cycle before onset of summer drought.

3. Differences in phenology among species require specific grazing management. Safe grazing can commence following the initiation of the axillary leaf. Stock must be removed from paddocks at the time of first flower to allow seed set. This closing date differs among species: July for gland clover, September for balansa and arrowleaf clovers and November for Persian clover. When clovers have reached physiological maturity, resume normal grazing from late October for gland clover, late November for balansa clover and late December for both arrowleaf and Persian clovers. These dates are recommended for crops sown in late February/early March. Hard graze in February to produce bare ground for seedlings re-establishment.

4. Early flowering gland clover is best suited in areas that dry out at the end of October or early November. Mid-flowering balansa clover and its ability to tolerate water logging may have a place in sites that experience wet winter and dry summer. Late flowering arrowleaf and Persian clovers are suitable in areas receiving high spring rainfall. Because arrowleaf clover does not tolerate water logging, this species should be grown on well

drained sites while Persian clover can be grown on sites that commonly experience water logging in winter.

5. The ability of an annual pasture crop to extend its dry matter production is dependent on its duration of growth. This highlights a trade-off between development and growth. Autumn sown crops had slower development thus required longer time to complete its life cycle. The advantage is that they can use this long growing season to maximise their dry matter production. In contrast, later sown crops flower quickly and mature before they could produce much.

6. On-going contribution to pasture system not only depends on production of seeds in the establishment year but also on the ability of seeds to spread their germination across time and the dynamics of the seed bank.

7. The ability of annual clovers to re-establish from seeds on soil surface indicates their potential for introduction to oversown areas.

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Appendices

Appendix 1 The coefficients and standard error for the linear regression between the germination rate and constant temperatures at sub-optimal and supra-optimal range for four annual clovers sown in incubators at Lincoln University, New Zealand.

	Sub-optimal (T_b-T_{opt})					Supra-optimal (T_{opt}-T_{max})				
Species	a	s.e.	b	s.e.	R^2	a	s.e.	b	s.e.	R^2
A	-0.045	0.0180	0.0330	0.00149	94	1.387	0.1660	-0.0399	0.00614	92
B	-0.103	0.0312	0.0392	0.00257	96	1.600	0.1640	-0.0472	0.00606	87
G	0.004	0.0328	0.0348	0.00311	97	1.081	0.1270	-0.0317	0.00492	70
P	-0.236	0.0923	0.0526	0.00507	90	5.230	1.3500	-0.1151	0.03600	75

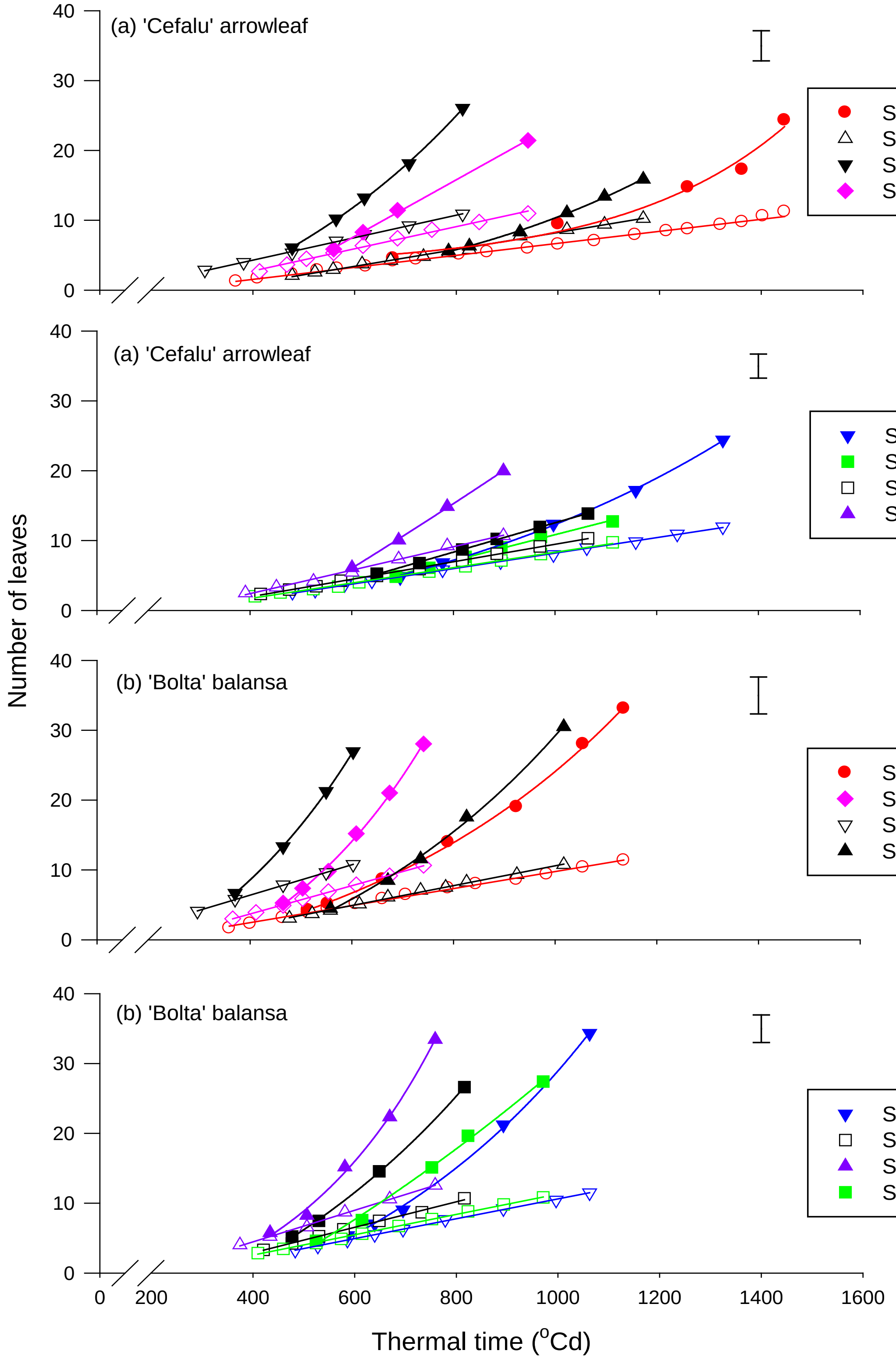
A., ‘Cefalu’ arrowleaf; B., ‘Bolta’ balansa., G., ‘Prima’ gland; P., ‘Mihi’ Persian. a, y-intercept; b, slope. s.e., standard error., R^2 unit is in percentage.

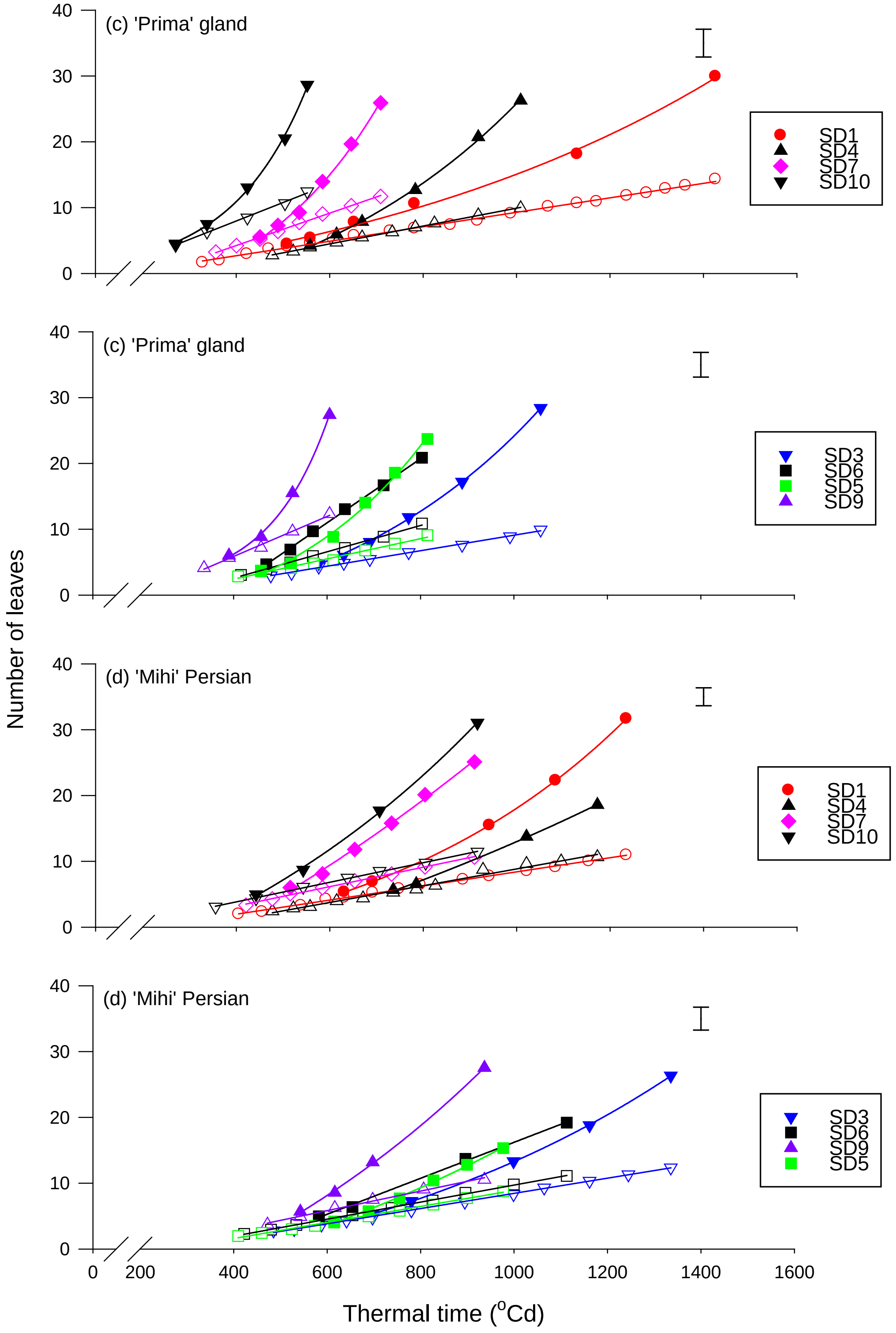
Appendix 2 The coefficients and standard error for the linear regression between the rate of emergence and mean soil (10 mm) temperature for four annual clovers sown in Iversen 9 field, Lincoln University, New Zealand.

Species	Temperature range (^oC)	a	s.e.	b	s.e.	R^2
A	5.3 -19.1	-0.011	0.0133	0.0122	0.00111	82
B	5.1 -10.4	0.032	0.0093	0.0069	0.00124	67
	12.1 - 18.8	-0.194	0.1400	0.0269	0.00879	43
G	5.2 - 18.8	0.002	0.0158	0.0103	0.00132	69
P	5.2 - 12.1	0.016	0.0115	0.0090	0.00133	70
	16.1 - 18.5	-0.554	0.4760	0.0519	0.02750	27

A., ‘Cefalu’ arrowleaf; B., ‘Bolta’ balansa., G., ‘Prima’ gland; P., ‘Mihi’ Persian. a, y-intercept; b, slope. s.e., standard error., R^2 unit is in percentage.

Appendix 3 Number of total (closed symbols) and main stem (open symbol) leaves of (a) ‘Cefalu’ arrowleaf, (b) ‘Bolta’ balansa, (c) ‘Prima’ gland and (d) ‘Mihi’ Persian clover plotted against thermal time after sowing. Error bars represent the maximum standard error for the final total leaf number.




(c) 'Prima' gland
SD1
SD4
SD7
SD10
(c) 'Prima' gland
SD3
SD6
SD5
SD9
(d) 'Mihi' Persian
SD1
SD4
SD7
SD10
(d) 'Mihi' Persian
SD3
SD6
SD9
SD5
Number of leaves
0
10
20
30
40
0
200
400
600
800
1000
1200
1400
1600
Thermal time (^{o}Cd)

Appendix 4 Coefficients of fitted relationship between thermal time to flowering (^oC d) and direction of photoperiod (hours) change at emergence in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	y = 1201 - 15.7x	30
	Decrease into autumn	y = 5807 - 288.7x	96
	Decrease into winter	y = -835 + 200.1x	90
'Bolta' balansa	Increase	y = 1408 - 48.2x	90
	Decrease into autumn	y = 6900 - 378.1x	99
	Decrease into winter	y = -1056 + 206.9x	97
'Prima' gland	Increase	y = 1287 - 52.0x	91
	Decrease into autumn	y = 4315 - 231.3x	96
	Decrease into winter	y = -1458 + 226.3x	97
'Mihi' Persian	Increase	y = 2571 - 113.2x	85
	Decrease into winter	y = -1408 + 293.6x	99

R^2, coefficient of determination.

Appendix 5 Coefficients of fitted relationship between thermal time to flowering (^oC d) and direction of photoperiod (hours) change at first (spade) leaf stage in 'Cefalu' arrowleaf, 'Bolta' balansa, 'Prima' gland and 'Mihi' Persian clover.

Species	Direction of photoperiod	Relationship	R^2 (%)
'Cefalu' arrowleaf	Increase	y = 1240 – 18.1x	37
	Decrease into autumn	y = 5409 – 266.7x	98
	Decrease into winter	y = -222 + 157.1x	85
'Bolta' balansa	Increase	y = 1469 – 51.4x	92
	Decrease into autumn	y = 6437 - 352.1x	100
	Decrease into winter	y = -687 + 182.8x	96
'Prima' gland	Increase	y = 1338 – 54.2x	94
	Decrease into autumn	y = 4047 - 216.3x	94
	Decrease into winter	y = -943 + 187.2x	
'Mihi' Persian	Increase	y = 2660 – 115.8x	93
	Decrease into winter	y = -1024 + 271.0x	98

R^2, coefficient of determination.

Appendix 6 Dates of flowering (Stage 1) in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown at ten different dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

	Species			
SD	Cefalu' arrowleaf	Bolta' balansa	Prima' gland	Mihi' Persian
26/2/2010	21/9/2010	14/9/2010	16/7/2010	16/11/2010
30/3/2010	16/10/2010	1/10/2010	9/9/2010	20/11/2010
4/5/2010	23/10/2010	8/10/2010	22/9/2010	20/11/2010
3/6/2010	24/10/2010	15/10/2010	28/9/2010	22/11/2010
7/7/2010	8/11/2010	22/10/2010	12/10/2010	23/11/2010
14/8/2010	19/11/2010	8/11/2010	29/10/2010	3/12/2010
25/9/2010	16/12/2010	6/12/2010	20/11/2010	16/12/2010
9/11/2010	20/1/2011	31/12/2010	25/12/2010	5/2/2011
20/12/2010	5/3/2011	10/2/2011	7/2/2011	-
19/1/2011	29/4/2011	31/3/2011	9/3/2011	-

Each date of flowering is means of four replicates.

Appendix 7 Dates of pollination (Stage 6) in ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers sown at ten different dates (SD) in Iversen 9 field, Lincoln University, Canterbury, New Zealand.

	Species			
SD	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’ Persian
26/2/2010	1/11/2010	17/10/2010	10/9/2010	30/11/2010
30/3/2010	19/11/2010	29/10/2010	14/10/2010	4/12/2010
4/5/2010	17/11/2010	2/11/2010	28/10/2010	4/12/2010
3/6/2010	30/11/2010	10/11/2010	29/10/2010	5/12/2010
7/7/2010	7/12/2010	10/11/2010	14/11/2010	8/12/2010
14/8/2010	17/12/2010	18/11/2010	15/11/2010	17/12/2010
25/9/2010	7/1/2011	15/12/2010	9/12/2010	25/12/2010
9/11/2010	16/2/2011	16/1/2011	7/1/2011	2/3/2011
20/12/2010	-	26/2/2011	21/2/2011	-
19/1/2011	-	-	-	-

Note: Cefalu’ arrowleaf sown on 20/12/10 and ‘Bolta’ balansa and gland clovers sown on 19/1/11 and ‘did not progress after stage 5. ‘Mihi’ Persian clover sown on 20/12/10 and 19/1/11 did not flower.

Appendix 8 Number of leaves on the main stem to axillary leaf appearance for ‘Cefalu’ arrowleaf, ‘Bolta’ balansa, ‘Prima’ gland and ‘Mihi’ Persian clovers validated at various locations in the South Island of New Zealand.

	Species			
Experiment location	‘Cefalu’ arrowleaf	‘Bolta’ balansa	‘Prima’ gland	‘Mihi’Persian
Control environment	5.7	4.7	7.0	4.5
Glasshouse nursery	5.0	4.8	4.8	n/a
Castle Hill	5.0	4.0	4.0	4.0
Lees Valley	5.0	4.9	4.8	4.0
Breach Oak	5.0	4.7	4.0	4.4
Maximum s.e.	0.17	0.11	0.48	0.15

n/a, not applicable; s.e., standard error.

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