Photoperiod affects the flowering time of field sown balansa clover

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Running head: Photoperiod affects flowering of balansa clover

Abstract

Two cultivars of balansa clover (*Trifolium michelianum* Savi.) were sown on eight occasions from 14 October 2005 to 5 February 2007 in Canterbury, New Zealand which gave a range of photoperiod at emergence between 8.6 and 15.7 hours. The duration from emergence to flowering was related to the length and direction of change in photoperiod at the time of emergence. Thermal time from emergence to flowering was constant at ~620 ^{o}Cd for 'Bolta' and ~365 ^{o}Cd for 'Frontier' balansa clover plants, provided they emerged into increasing photoperiods. For plants which emerged into decreasing photoperiods, thermal time from emergence to flowering decreased from ~1500 to ~630 ^{o}Cd for 'Bolta' but was constant at ~690 ^{o}Cd for 'Frontier'. Models are presented to predict the thermal time requirement from emergence to flowering for 'Bolta', 'Frontier' and, based on re-analysed data,

CPI45856 balansa clovers in relation to daylength at emergence. These results are discussed in relation to farm sowing and management practices.

Additional keywords: Crop model, hysteresis

Introduction

Balansa clover (*Trifolium michelianum* Savi.) is an aerial flowering Mediterranean annual legume that is grown in over 1.5 M ha in Australia (Craig and Ballard, 2000). When sown in a grazed pasture mixture with cocksfoot (*Dactylis glomerata* L,) at Lincoln University, New Zealand , balansa clover produced 30% of the 8-10 t DM/ha yield (Mills *et al.*, 2008). The dry matter production of balansa clover depended on regeneration from seed, itself dependent on the time of final spring grazing in relation to flowering date (Monks *et al.*, 2008). The time of flowering can therefore be used to assist management decisions on when to cease grazing to allow seed set and in some environments it determines which cultivar is most suitable. This contrasts the situation for subterranean clover where the self fertilized flowers are forced below ground and seeds are set even under continuous grazing (Ates *et al.*, 2008).

The time of flowering in many species is genetically controlled and responsive to temperature, photoperiod and the circadian rhythm (Evans, 1959). The plant aims to flower at the most advantageous time to set seed for regeneration. Many species delay the time of flowering if they are grown in environments with different photoperiod durations (Garner and Allard, 1920). The effects of photoperiod on the time of flowering have been quantified using photothermal time as defined by Weir *et al.* (1984). This method has been used to predict flowering time in many crops

(Summerfield *et al.*, 1991) including wheat (Porter *et al.*, 1987) and subterranean clover (Evans *et al.*, 1992). In most cases analyses have been based on the mean photoperiod experienced by the plant from emergence to flowering. However, predicting flowering date using the mean photoperiod from emergence to flowering is circuitous because it requires prior knowledge of the flowering date to be predicted. In response, Brooking *et al.* (1995) suggested that the length and direction of change of the photoperiod at a single point in the life of a plant could be used to predict the date of flowering.

This study was conducted to test the hypothesis that photoperiod and temperature can be used to quantify the time of flowering for two balansa clover cultivars. This was done through a field experiment where analyses were compared with published literature to define the drivers of time to flowering for balansa clover cultivars. Accurate prediction of the time of flowering would assist on-farm management decisions regarding the time of animal grazing and seed production, and to determine the best cultivars to sow and the optimum sowing time for maximum herbage production.

Materials and Methods

This field experiment was conducted at Lincoln University, Canterbury, New Zealand (43^{o} 38'S, 172^{o} 28'E, 11 m a.m.s.l) on a Templeton silt loam soil (Udic Ustochrept, USDA Soil Taxonomy) with 1.8 – 2.5 m of fine textured material overlying gravels (Cox, 1978). These soils are imperfectly drained and display a strong mottling, which indicates periods of water logging. Sulfur superphosphate (8.7% P, 14.7% S, 19% Ca) at 200 kg/ha and urea (46% N) at 100 kg/ha were applied on 13 July 2005 following a soil test.

This experiment was a split-plot factorial in a randomised complete block design with three replicates and 48 plots. Main plots were 8 sowing dates (Table 1) and two balansa clover cultivars (‘Bolta’ and ‘Frontier’) as subplots. Before the first sowing, the area was cultivated to produce a firm, fine seedbed. Plots were also cultivated prior to each additional sowing.

Bare seed (from Agricom Ltd., Ashburton, New Zealand) was sown without inoculum in monocultures at 6 kg of viable seed/ha. The plots were 2.1 x 6.0 m and drilled in 150 mm rows at a target depth of ≤10 mm using an Øyjoord cone seeder followed by chain harrows. The plots were then Cambridge rolled.

All plots received 35 mm of irrigation water on both 13 and 20 April 2006 to ensure establishment and survival of seedlings. All sown plots were sprayed on 28 October 2006, after flowering, with Pulsar® (200 g/l bentazone and 200 g/l MCPB) at 5 l/ha and Gallant® (100 g/L haloxyfop) at 1.5 l/ha for the control of broadleaf and grass weeds (particularly *Poa annua*), respectively.

TABLE 1 APPROX HERE

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

Flower development was recorded at 2-5 day intervals from six marked plants per replicate from emergence. The time of flowering was defined as when 50% of plants had an inflorescence bud visible in the axil of a leaf. The node of first flower was recorded on 29 September 2006 for plants sown on 16 January, 8 March and 27 April.

Analysis

The time of flowering was analysed using thermal time and photothermal time. Thermal time was calculated daily as the mean of eight temperature values generated from maximum (MaxT) and minimum (MinT) temperatures using a function to model the sigmoid shape of temperature change through a day (Jones and Kiniry, 1986). Each of the values was calculated and then had base temperature subtracted before being averaged to give a single value of thermal time for each day. The model was of the form:

Equation 1 $$0.92105 + 0.114 \times I - 0.0703 \times I^2 + 0.0053 \times I^3$$

Where I = 1 through to 8. Base temperature, below which no development occurs (Angus *et al.*, 1981), was 2.5 ^{o}C and optimum temperature, at which development occurs at its maximum rate, was 15 ^{o}C. These values were derived from growth cabinet experiments to describe the vegetative development of balansa clover including, germination, emergence and leaf appearance (Monks, 2009).

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

Equation 2 $$Pt = FP \times Tt$$

Equation 3 $$FP = [(Pp - Pp_{base})/(Pp_{opt} - Pp_{base})]$$

where FP is a photoperiod modification factor, Pp is the photoperiod at emergence in hours, Pp_{opt} is the optimum photoperiod and the Pp_{base} is the base photoperiod (Summerfield *et al.*, 1991). Photoperiod was defined from sunrise to sun set, including civil twilight (Evans *et al.*, 1992). To define the optimum and base photoperiods an iterative least squares minimisation analysis was performed using values ranging from 0 to 24 hours. This analysis showed that for this long day plant setting the optimum photoperiod at 15.7 hours and the base photoperiod at 8.6 hours, which correspond to the maximum and minimum photoperiods experienced at this latitude, gave the maximum R^2 of ~0.96 for both cultivars.

The node of first flower was analysed using Analysis of Variance (ANOVA) with means separation by least significant difference (LSD) (5%).

A second data set was generated from a reanalysis of flowering time for CPI45856 balansa clover sown near Kyabram airfield, northern Victoria, Australia (36^o 34'S, 145^o 06'E) (Kelly and Mason, 1986). Sowing dates for CPI45856 (treated as synonymous with emergence date) were 7 February, 28 February, 21 March and 11 April 1983. Final emergence percent was low at 18% for the first two dates and this may have been due to high temperature dormancy in the seed (Monks *et al.*, 2009). Daily maximum and minimum temperatures for this dataset were generated from the monthly mean maximum and minimum temperatures for Kyabram airfield for 1983 (Anonymous, 2009) and fitted to the model in Equation 1. Standard errors, given in parentheses, were not able to be calculated for CPI45856.

Results

Calendar days from emergence to flowering

The number of days from emergence to flowering in 'Bolta' and CPI45856 decreased with each successive sowing date through the calendar year from January to December (Figure 1). For 'Frontier', the number of days to flowering was lower for plants sown between 16 January and 21 March than for plants sown on 27 April (Figure 1).

FIGURE 1 APPROX HERE

Thermal time from emergence to flowering

Thermal time was calculated to summarise the time from emergence to flowering within each cultivar. The thermal time required from sowing to flowering for 'Bolta' and CPI45856 exhibited a similar decline over time (Figure 2).

The accumulated thermal time for 'Frontier' decreased stepwise for plants sown after 27 April (Figure 2). Thus the total accumulated thermal time requirement for flowering for all three cultivars differed across sowing dates. To account for this systematic variation in flowering date, the influence of photoperiod was also examined.

FIGURE 2 APPROX HERE

Photoperiod at emergence

Thermal time from emergence to flowering formed a hysteresis with increasing and decreasing photoperiod at emergence for both 'Bolta' and 'Frontier' (**Error! Reference source not found.**). The sowing dates for CPI45856 resulted in plants only emerging into decreasing photoperiods (Kelly and Mason, 1986).

'Bolta'

All 'Bolta' balansa clover plants that emerged after the longest day (21 December) flowered in September, when photoperiod was between 11 and 12.3 hours. However, the number of thermal units from sowing to flowering decreased for plants emerging between 16 January and 3 July (**Error! Reference source not found.**). The relationship found was a 148 (±5.1) ^{o}Cd decrease for each hour reduction in daylength at emergence (R^2 = 0.99) from 1500 ^{o}Cd on 16 January to 630 ^{o}Cd on 7 July.

During this period, the nodes at first flower of 'Bolta' balansa clover decreased ($P<0.001$) from 14.5 to 10.1, mirroring the decrease in time to flowering as sowing date progressed from 16 January to 7 July. In contrast, the number of thermal units required from emergence to flowering remained relatively constant, decreasing by 8 (±7.2) ^{o}Cd for each hour increase in daylength at emergence (R^2 = 0.52).

'Frontier'

For 'Frontier' balansa clover seedlings, the number of thermal units from emergence to flowering regressed against photoperiod at emergence also formed a hysteresis.

Flower initiation required a relatively constant 690 °Cd as daylength decreased at emergence and 365 °Cd as daylength increased (**Error! Reference source not found.**). As daylength decreased at emergence, each hour increased thermal time requirement by 7 (±13.2) °Cd (R^2 = 0.13). As daylength increased at emergence, each hour thermal time requirement increased by 12 (±2.1) °Cd (R^2 = 0.97).

During this period, the node at first flower decreased (P<0.001) from 9.2 for plants sown on 16 January to 8.2 for those sown on 8 March. The node of first flower then remained constant at 8.2 for plants sown on 27 April.

Reanalysis of Kelly and Mason (1986)

Thermal time from emergence to flowering was also calculated for CPI45856 balansa clover from five dates between 7 February and 20 June 1982. CPI45856 is a parent of 'Paradana', itself a parent of 'Frontier' (Kelly and Mason, 1986; Craig *et al.*, 2000), but the line exhibited the same response to photoperiod as 'Bolta'. All CPI45856 plants were sown and emerged into decreasing photoperiods and flowered between 20 and 28 September, when photoperiod was ~12 hours. This relationship showed that in decreasing photoperiod each additional hour of daylength at emergence raised the thermal time to flower by 239 (±28.1) °Cd (R^2 = 0.96).

FIGURE 3 APPROX HERE

Photothermal time

To unify the flowering time from any given sowing date, based on the photoperiod at emergence, the amount of thermal time accumulated between emergence and flowering was modified by photoperiod (Weir *et al.*, 1984). The photothermal time model reduces the thermal time accumulated each day through the use of a base photoperiod, in this case 8.6 hours. There was a single linear function (Figure 3) between photothermal time and the photoperiod at emergence for both cultivars. Each additional hour of daylength at emergence increased the photothermal time requirement to flowering by 64 (±16.3) ^{o}Cd for 'Bolta' ($R^2 = 0.79$) and by 57 (±9.5) ^{o}Cd for 'Frontier' ($R^2 = 0.88$).

However, this approach did not satisfactorily account for the differences in flowering time when plants were sown immediately before and after the longest day (1 December and 16 January). Plants which emerged into similar photoperiods but on either side of the longest day had a 160 ^{o}Cd difference in time to flowering.

FIGURE 4 APPROX HERE

Discussion

The time of flowering for balansa clover was quantified by thermal time and modified by the length and direction of change of the photoperiod at emergence. Previous relationships between plant flowering and day length, that utilize long and short day plant classifications (Major, 1980) or photothermal time (Summerfield *et al.* 1991), were unable to capture the variation in time of flowering as well as the straight thermal time model. The relationship was strongest for each cultivar when the model assumed the amount of time required from emergence to flowering was based on the duration and direction of photoperiod at emergence. This implies the plants somehow perceived daylength, possibly by comparison of successive days or with an 'expectation' derived from an endogenous measure of circadian rhythm (Millar, 2004).

In a Mediterranean environment, where balansa clover originates, photoperiod increases occur in conjunction with increased temperatures and decreased moisture availability (Craig, 1998; Craig *et al.*, 2000; del Pozo and Aronson, 2000). Therefore, photoperiod-responsive pathways which induce flowering more swiftly (shorter thermal time) as photoperiod increases would be a positive survival mechanism. The consequence would be a pre-disposition to produce seeds prior to death by drought. In these environments, plant survival is promoted by the ability to produce reproductive structures sooner than would be necessary in more favourable conditions (Norman *et al.*, 2005).

These results provide a physiological basis for traditional sowing date and cultivar choice recommendations for balansa clover. Choosing the correct time to sow will depend on the date in relation to the longest day, the soil temperature and moisture status. 'Bolta' and CPI45856 balansa clovers had their longest duration of vegetative growth when sown immediately after the longest day. Therefore, the greatest dry matter production possible for a growing season would come from the earliest practical sowing. This was shown by Kelly and Mason (1986) for CPI45856 sown in northern Victoria. In contrast, the duration of the vegetative period for 'Frontier' balansa clover was unaffected by sowing date and therefore it is less able to take advantage of any favourable autumn growing conditions without shortening the duration of spring growth.

Soil temperature at sowing is also considered important for successful establishment. The high temperatures of summer associated with dates immediately following the longest day lead to temperature-induced seed dormancy (Jansen and Ison, 1994; Monks *et al.*, 2009). ‘Bolta’ and ‘Frontier’ have both been shown to germinate to their maximum amount at temperatures below 20 ^{o}C and at their maximum rate between 12 and 14 ^{o}C (Monks *et al.*, 2009). In temperate environments, autumn rains tend to coincide with a decrease in temperatures. Sowing should be timed to ensure sufficient moisture for emergence and to avoid seedling death by early season drought (false break).

Cultivar choice is dependent on the length of the growing season to maximise vegetative growth and ensure sufficient seed is set for the following year. This work confirms the conventional sowing recommendations made by Wurst *et al.* (2004) that ‘Bolta’ should be sown in environments where the season typically ends in November compared with ‘Frontier’ for regions with a shorter season (~September). Sowing in early February to maximise dry matter production may require a spring/summer fallow or irrigation to ensure adequate soil moisture. The potential would then be for high quality dry matter to be grazed in March/April, before winter slows growth. Removing grazing animals in early August, for ‘Frontier’, or September, for ‘Bolta’, would allow the plants to set large amount of seed (Monks *et al.*, 2008).

Where variable spring moisture may limit the duration of the growing season, cultivars with different flowering dates may be sown together to maximise dry matter production while ensuring some seed is set. Provided seed set occurs every four years adequate plant populations are likely to be maintained (Monks *et al.*, 2008).

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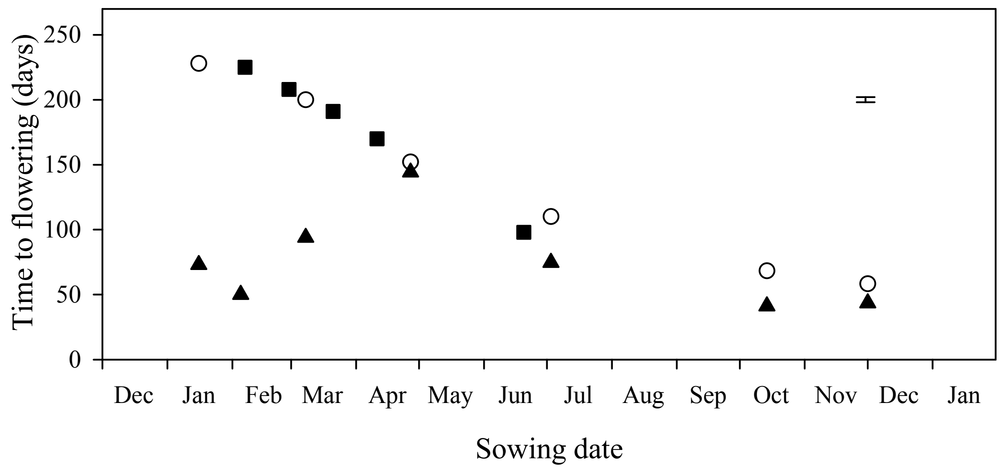


Figure 1

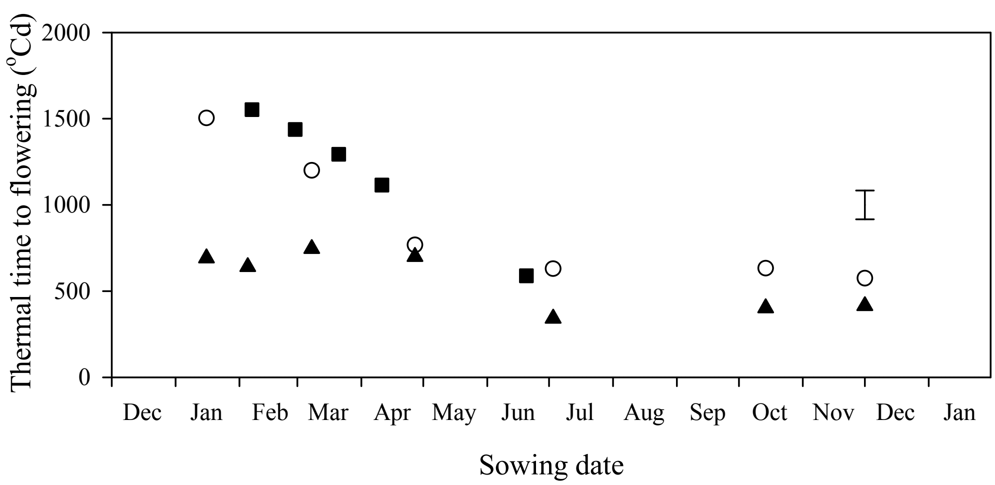
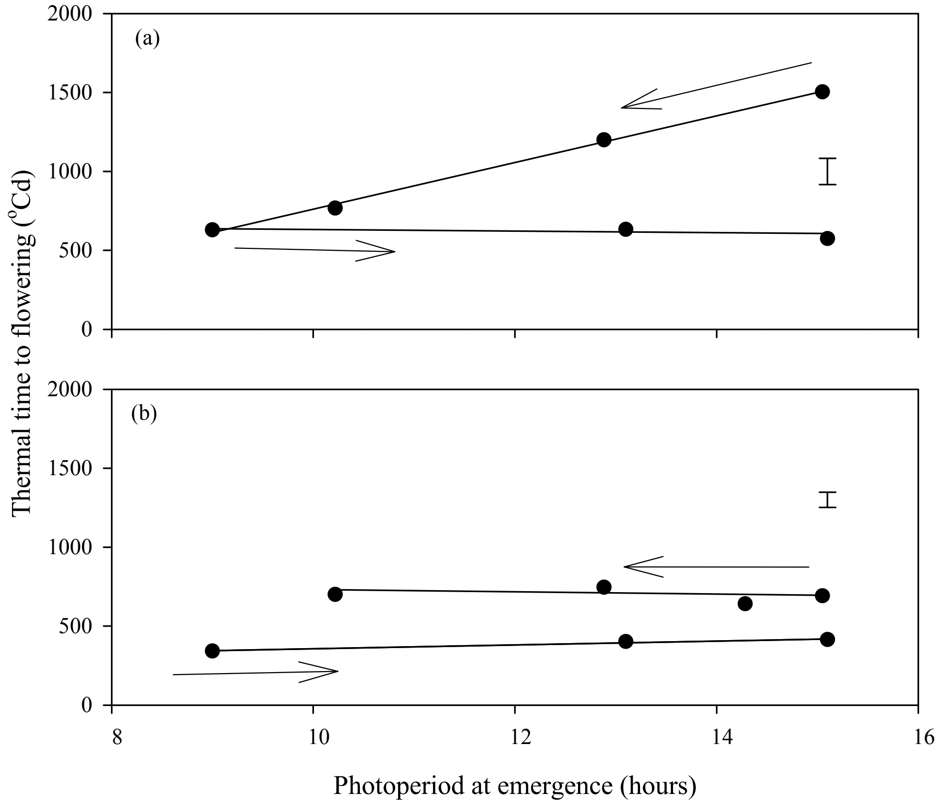


Figure 2



(a)
(b)
2000
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1000
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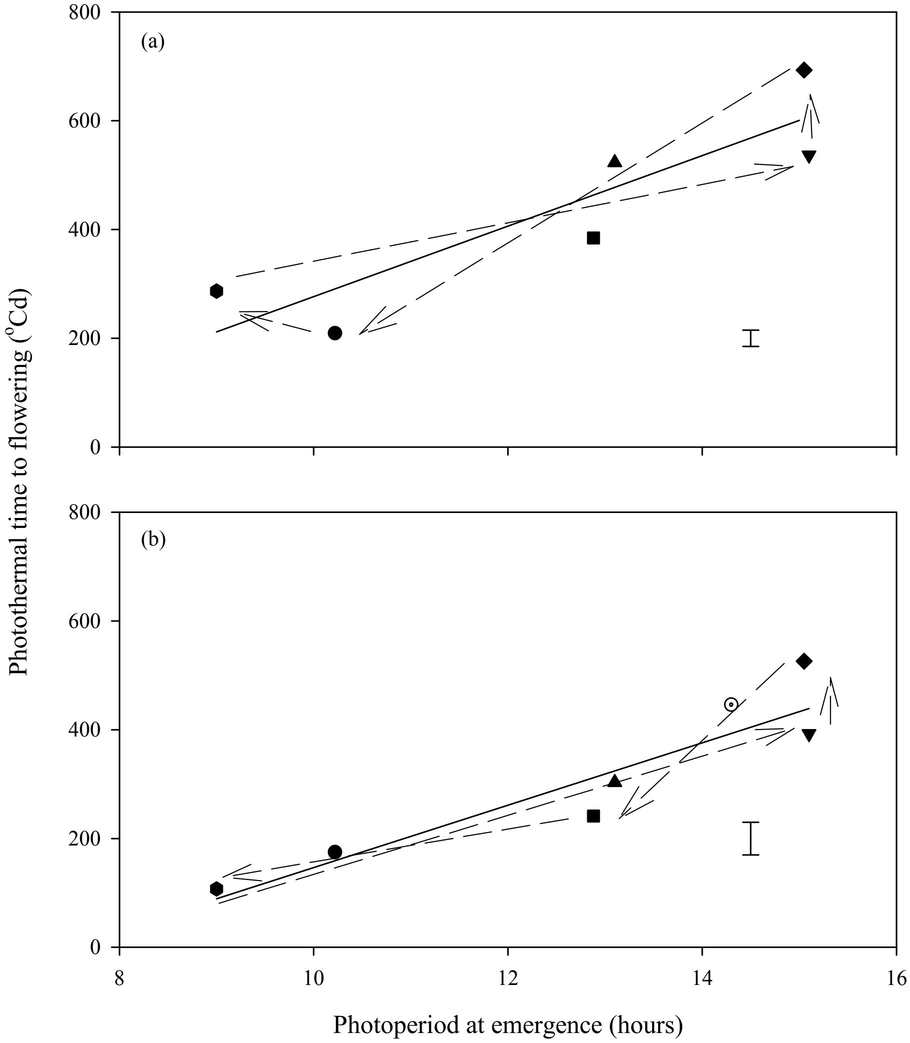
10
12
14
16
Thermal time to flowering (°Cd)
Photoperiod at emergence (hours)

Figure 3


(a)
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Photothermal time to flowering (^oCd)
Photoperiod at emergence (hours)

Figure 3

Figure 1 caption:

Number of days from emergence to flowering for ‘Bolta’ (○) and ‘Frontier’ (▲) balansa clover sown in Canterbury, New Zealand and CPI45856 (■) sown in Kyabram, Australia.

Note: Error bars show maximum standard error.

Figure 2 caption:

Thermal time from emergence to flowering for ‘Bolta’ (○) and ‘Frontier’ (▲) balansa clover sown in Canterbury, New Zealand and CPI45856 (■) sown in Kyabram, Australia.

Note: T_{base} = 2.5 ^{o}C. Error bars show maximum standard error.

Figure 3 caption:

Thermal time from emergence to flowering against photoperiod at emergence for (a) 'Bolta' and (b) 'Frontier' balansa clover, sown in Canterbury, New Zealand. Lines show response to either decreasing or increasing photoperiod (indicated by arrows). Error bars show maximum standard error.

Figure 4 caption:

Regression of photothermal time to flowering against photoperiod at emergence for (a) 'Bolta', and (b) 'Frontier' balansa clover sown in Canterbury, New Zealand on 14 October (▲), 1 December (▼), 16 January (◆), 5 February (⊙), 8 March (■), 27 April (●), 3 July (⬢). Arrows show the direction of time for either decreasing or increasing photoperiod at emergence. Error bars show maximum standard errors.