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# Leaf appearance of annual clovers responds to photoperiod at emergence

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ABSTRACT

Leaf appearance rate and time to canopy expansion of four annual clover species (arrowleaf, balansa, gland and Persian) were quantified in field and controlled environment studies. Crops sown in autumn, which experienced shortening daylengths at emergence, had a slower rate of leaf production and consequently took a longer time to initiate branching, than spring-sown crops. When autumn-sown 'Bolta' balansa clover emerged on the shortest day in winter (21 June), the rate of leaf appearance was lengthened by 4 °C d/leaf/h. When the same species emerged after the shortest day, into an increasing photoperiod, the phyllochron was shortened by 5 °C d/leaf/h. This influence of photoperiod on the phyllochron consequently altered the time to axillary leaf production (branching). Throughout all sowing dates, phyllochron was the fastest for 'Prima' gland (33–91 °C d/leaf) and slowest for 'Cefalu' arrowleaf (53–116 °C d/leaf) clovers. 'Bolta' balansa was 44–82 °C d/leaf and 'Mihi' Persian 61–93 °C d/leaf. The response of phyllochron to photoperiod suggests these annual clovers should be sown in late summer or early autumn to initiate axillary leaf production as soon as possible to ensure maximize dry matter for early spring.


## 1. Introduction

In most annual crops, the rate of leaf appearance (phyllochron) and then their expansion on the primary stem affects canopy expansion and can influence the time to first flower. Predominantly, research on annual crops has focussed on leaf appearance in the major cereals and leaf appearance has been used as a descriptor to quantify the time to flowering (Jamieson et al., 1995; Sonego, 2000). This is because anthesis marks the switch from vegetative to reproductive development and the duration of grain filling commences at anthesis. Therefore, yield predictions in many computer simulation models are based on predicting flowering dates and the accumulation of thermal time post-anthesis.

For annual pasture clovers, the emphasis has also been on predicting time of flowering (Evans, 1959; Evans et al., 1992) because this marks the initiation of seed set which is essential for regeneration of the species in subsequent years. A recent study showed that floral initiation of annual clovers occurred after a specific number of nodes appeared on the main stem, and that this phenology differed with the time of planting (Nori et al., 2014). Flower induction was predominantly controlled by temperature and modified by photoperiod at seedling emergence. However, for annual forage crops, leaf appearance is also important for consumption and competitive ability in establishment of mixed pasture swards.

Specifically, the time to the first axillary leaf production (branching) is a key development phase because it indicates the time when the number of leaves and total leaf area increases exponentially. The canopy of leaves then allows the pasture species to compete strongly for light. The success or failure of a species can depend on the time taken to initiate branching. For example, the extended time required for Caucasian clover (*Trifolium ambiguum*) to produce its first axillary leaf (1180 °C d) compared with the 440 °C d for white clover (*Trifolium repens*) and 373 °C d for perennial ryegrass (*Lolium perenne*) means it is a poor competitor in a sward. This has limited its adoption as a pasture species (Black et al., 2006).

Leaf appearance rate is often reported to be consistent irrespective of the time of planting (Hotsonyame and Hunt, 1997; Jamieson et al., 1995; Miglietta, 1989; Slafer and Rawson, 1997) and to be solely driven by the temperature at the growing point (Peacock, 1975). Jamieson et al. (1995) suggested that discrepancies of phyllochron with sowing dates were due to a mismatch between the measured temperature and the site of temperature perception. Other researchers suggest that inconsistencies of leaf appearance rate with time of sowing were attributed to photoperiod at the location of planting (Baker et al., 1980; Brown et al., 2005; Sonego,

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**Table 1**
Sowing dates and photoperiod at seedling emergence (h) used to quantify leaf appearance of four annual clover species sown at Lincoln University, Canterbury, New Zealand.

| Sowing no. | Sowing date | Photoperiod at emergence (h) |
|---|---|---|
| 1 | 26 February 2010 | 13.7 |
| 2 | 30 March 2010 | 12.0 |
| 3 | 4 May 2010 | 10.7 |
| 4 | 3 June 2010 | 10.0 |
| 5 | 7 July 2010 | 10.5 |
| 6 | 14 August 2010 | 11.8 |
| 7 | 25 September 2010 | 13.7 |
| 8 | 9 November 2010 | 15.7 |
| 9 | 20 December 2010 | 16.6 |
| 10 | 19 January 2011 | 15.9 |

2000). These researchers found that the phyllochron responded to the direction and change in photoperiod. Plants that grew during a lengthening photoperiod had a shorter phyllochron than those in a declining photoperiod. These studies on temperature and photoperiod effects have predominantly been tested on wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lucerne (*Medicago sativa*) and oat (*Avena sativa*), but not annual clovers. Monks (2009) estimated a single phyllochron of 47 °C d/leaf for 'Bolta' balansa clover (*Trifolium michelianum*) across six sowing dates in a field experiment at Canterbury, New Zealand. However, the relationship between phyllochron and photoperiod was not analysed due to the limited range of sowing dates. In addition, leaf appearance of other species of annual clovers has not been studied extensively. Therefore, this study aimed to determine phyllochron and time to first axillary leaf production (branch initiation) of four species of annual clovers sown at 10 dates in a field experiment in Canterbury, New Zealand in 2010–2011.

## 2. Materials and methods

The major field experiment was located in Iversen Field at Lincoln University, Canterbury, New Zealand (43° 38′S, 172° 28′E, 11 m a.s.l.). Models that correlate between phenology and temperature and photoperiod were generated from this set of field experiments. To determine the accuracy of these derived models in prediction of phyllochron and branch initiation over a wide range of environments, the same four species (Section 2.1) were sown in a controlled environment chamber (CEC) and in pots in a nursery at Lincoln University, Canterbury. Additional field observations occurred on commercial farms at (1) Castle Hill, Canterbury (43° 14′S, 171° 43′E, 700 m a.s.l.), (2) Lees Valley, Canterbury (43° 8′S, 172° 11′E, 434 m a.s.l.), and (3) Breach Oak, Marlborough (41° 43′S, 173° 58′E, 198 m a.s.l.) in the South Island of New Zealand.

### 2.1. Major field experiments in Lincoln University, Canterbury

A split-plot design experiment with four replicates was sown on 10 dates (Table 1). The main plots were sowing dates, with the four annual clover species; 'Cefalu' arrowleaf (*T. vesiculosum*), 'Bolta' balansa (*T. michelianum*), 'Prima' gland (*T. glanduliferum*) and 'Mihi' Persian (*T. resupinatum*) as the subplots. Each species was sown as a monoculture at 6 kg/ha for 'Cefalu' arrowleaf, 4 kg/ha for 'Bolta' balansa and 'Prima' gland and 5 kg/ha for 'Mihi' Persian clovers. Plots were not grazed or cut throughout the experimental period. A full description of the experimental site, design and management were reported previously by Nori et al. (2014).

For each sowing date, the number of leaves that appeared on the primary 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. The appearance of the first axillary leaf (branch initiation) was determined as the point when the number of total leaves exceeded the number of primary stem leaves by one.

### 2.2. Pot experiments in controlled environment chamber (CEC).

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 scarified bare seeds of the four species (Section 2.1) with four replicates were placed on the potting mix. These pots were arranged in a completely randomized design in a CEC (Conviron PGV36, Winnipeg, Canada) at one of five programmed temperature regimes: 9/6, 15/6, 20/10, 25/15 and 30/20 °C. Both temperature and photoperiod were set at 8/8 h with 4 h transitions between light and dark. This gave a constant 16 h daily photoperiod within the CEC. The chamber was lit with a combination of 45 incandescent (Sylvania, 40 W) and 30 fluorescent (Sylvania, 6 × 115 W and 24 × 215 W) lamps. This environment created a photosynthetic photon flux density (PPFD) of 448 ± 9 μ moles/m$^2$/s at the plant canopies. Relative humidity ranged between 50 to 70%.

Three temperature sensors (Thermistors KTY-110) were buried at 10 mm depth and one placed at ∼0.4 m above ground level, protected by an aluminium tube. Temperatures were recorded every 15 min with a HOBO data logger (Onset Computer Corporation) to determine daily mean temperatures. 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.

### 2.3. Pot experiments in the nursery, Lincoln University

The aim of the pot experiments in the Lincoln University nursery was to produce plants at times not covered in the field experiment. Thus, 'Bolta' balansa clover was sown on 24 January 2012, while 'Cefalu' arrowleaf and 'Prima' gland clovers were sown on 7 February 2012. Seeds were sown at 11 kg/ha for 'Cefalu' arrowleaf and 7 kg/ha for both 'Bolta' balansa and 'Prima' gland clovers in a plastic container (460 × 300 × 100 mm) filled with bark and pumice potting mix (Section 2.2). All pot experiments were established on the ground in the open air. A temperature sensor was placed at 400 mm above the ground to measure daily maximum and minimum air temperatures. 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. In each pot, 10 plants were marked for detailed measurements of vegetative development.

**Table 2**
Daily mean air temperature, monthly rainfall and soil type at Castle Hill and Lees Valley, Canterbury and Breach Oak, Marlborough, New Zealand in 2011.

| Location | Daily mean air temperature (°C) | Monthly rainfall (mm) | Soil type |
|---|---|---|---|
| Castle Hill, Canterbury | 1 °C (July)–14 °C (January) | 23 mm (September)–120 mm (July) | Craigieburn silt loam (D.S.I.R., 1968) |
| Lees Valley, Canterbury | 3 °C (July)–16 °C (January) | 37 mm (September)–140 mm (October) | Ashwick shallow silt loam (D.S.I.R., 1968) |
| Breach Oak, Marlborough | 5 °C (July)–18 °C (February) | 0.6 mm (January)–97 mm (May) | Flaxbourne silt loam (D.S.I.R., 1968) |