Vegetative development of four annual clovers

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Abstract
A field experiment with 10 sowing dates quantified thermal time requirement for seedling development of arrowleaf, balansa, gland and Persian clovers. A base temperature (T_b) of 0^oC was found for all species. Balansa and Persian clovers required ~213^oC d from sowing to first leaf appearance compared with 231 ^oC d for arrowleaf and 221 ^oC d for gland clover. The phyllochron for these annual clovers was affected by photoperiod at emergence. For example, the phyllochron for balansa clover was 82 ^oC d when plants emerged into a 13.6 hour with decreasing photoperiod but only 44 ^oC d when plants emerged into a 15.6 hour with increasing photoperiod. These differences in phyllochron also affected the time of axillary leaf appearance. A slower phyllochron resulted in longer time to the first axillary leaf.

Key Words
Leaf appearance, *Trifolium glanduliferum, T. michelianum, T. resupinatum, T. vesiculosum*

Introduction
Annual clovers are used in dryland pastures to provide sheep grazing during late winter and early spring. Subterranean clover (*Trifolium subterraneum* L.) is the most widely used annual legume species in New Zealand. However, its seed burr means specialised machinery that can cause environmental damage is required for seed harvest, and none is currently produced commercially in New Zealand. Alternativley, top flowering species such as arrowleaf (*Trifolium vesiculosum* Savi), balansa (*T. michelianum* Savi), gland (*T. glanduliferum* Boiss.) and Persian (*T. resupinatum* L.) clovers may have potential for use in dryland pastures (Charlton and Stewart 2003; Dear *et al.* 2002; Evans and Mills 2008; Monks *et al.* 2008). These top flowering annual clovers are not widely used in New Zealand and little is known about the performance of these species under New Zealand conditions. Developmental characteristics in seedlings partially determine their success in a pasture sward. Specifically, leaf appearance rate (phyllochron) and timing of first axillary leaf affect canopy development and the consequent competitiveness of seedlings. Development processes such as leaf appearance can be quantified in thermal time (Tt).

Thus, the objective of this study was to quantify thermal time requirements for vegetative development of arrowleaf, balansa, gland and Persian clovers and relate this to temperature and daylength as the main drivers of seedling development.

Methods
The experiment was established at Lincoln University, Canterbury, New Zealand (43^o 38'S, 172^o 28'E, 11 m a.m.s.l.) on a Wakanui silt loam (Cox 1978) soil. A split-plot design with four replicates was initiated on 10 sowing dates (main plots) with four annual clover species ('Cefalu' arrowleaf, ('Bolta' balansa, 'Prima' gland and 'Mihi' Persian) sown in 6 m^2 subplots.. The 10 sowing dates were: 26 Feb 10 (SD1), 30 Mar 10 (SD2), 4 May 10 (SD3), 3 Jun 10 (SD4), 7 Jul 10 (SD5), 14 Aug 10 (SD6), 25 Sep 10 (SD7), 9 Nov 10 (SD8), 20 Dec 10 (SD9) and 19 Jan 11 (SD10). Bare seeds of clover species were 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. All seeds were sown with Group C inoculants (ALOSCA Tech. Ltd.). Soil (10 mm depth) and air (1200 mm above ground) temperature were recorded hourly using a HOBO data logger to define daily maximum and minimum temperatures for thermal time calculations. Daily photoperiod was determined from geographic coordinates and includes civil twilight. Three 0.01m^2 quadrats were placed in a fixed position on each subplot. The number of seedlings with spade leaf appearance was counted every alternate day until all seedlings within the quadrat had produced a leaf. The number of days to reach 50% of final first leaf appearance (t_{50}) was determined from the Gompertz model, t_{50}= M-ln[-ln(y/100)]/B where, M and B are constants. The base temperature and thermal time requirements for first leaf were defined using a linear model of development rate ($1/t_{50}$) against mean temperatures as described by Angus *et al.* (1981) and Moot *et al.* (2000). The number of emerged leaves on the main stem and appearances of axillary buds were counted at 4-7 day intervals on 10 marked plants per subplot. The phyllochron was calculated from least squares regression of the number of leaves against thermal time accumulation across sowing dates and then

analysed in relation to photoperiod at emergence. Quantification of thermal time to first leaf used soil temperature while phyllochron and axillary leaf initiation used air temperature based on the position of plant's growing point (Jamieson *et al.* 1995).

Results and discussion

In each species, the number of days to first leaf appearance was ~41 days at the lowest temperature of 5.3°C (SD4) and then decreased with every increment of mean soil temperature (~11 days at 18.6°C (SD8)). In all species, T_b calculated was not different from 0°C. With T_b set to 0°C, to enable comparison among species (Moot et al. 2000), the Tt requirement for first leaf appearance averaged 213(±9.4) °C d for both balansa and Persian clovers compared with 221(±11.6) °C d for gland and 231(±6.4) °C d for arrowleaf clovers. There was an interaction (P<0.001) between sowing date and species for the phyllochron (Table 1). The phyllochron exhibited a declining trend following each successive sowing date with the longest phyllochron from pastures sown on 26 February 2010 and the shortest for those sown on 9 November 2010.

Table 1. The phyllochron (°C d/leaf) of arrowleaf, balansa, gland and Persian clovers sown on different dates in Lincoln University, Canterbury, New Zealand.

Sowing date (SD)	Annual clover species: arrowleaf	balansa	gland	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°C) and calculated starting from first leaf appearance.
SD, Sowing date. S.E.M. Standard error of mean; L.S.D., Least significant differences.

The phyllochron responded to changes in the length and direction of photoperiod at seedling emergence. When seedlings emerged following the shortest day (21 June) into an increasing photoperiod up to 16 hours, the phyllochron decreased by 6 (±0.9) °C d leaf^{-1} hour^{-1} for arrowleaf, 5 (±0.9) °C d leaf^{-1} hour^{-1} for balansa, 7 (±1.1) °C d leaf^{-1} hour^{-1} for gland and 3 (±0.6) °C d leaf^{-1} hour^{-1} for Persian clover. Following the longest day, as photoperiod shortened to ~13 hours into the autumn, phyllochron rose rapidly by 21 (±2.6) °C d leaf^{-1} hour^{-1} for both arrowleaf and gland, 14 (±1.4) °C d leaf^{-1} hour^{-1} for balansa and 10 (±1.0) °C d leaf^{-1} hour^{-1} for Persian clover. However, as photoperiod continued to decrease further into the winter, the phyllochron decreased by 9 (±1.5) °C d leaf^{-1} hour^{-1} for arrowleaf , 4 (±0.9) °C d leaf^{-1} hour^{-1} for both balansa and gland and 7 (±0.9) °Cd leaf^{-1} hour^{-1} for Persian clover.

The first axillary leaf was initiated in the axil of the spade leaf in balansa and Persian clovers and in the axil of the first trifoliate leaf in arrowleaf and gland clovers. For most sowing dates, the initiation of axillary leaf began after the appearance of four leaves on the main stem of balansa, gland and Persian clovers and five leaves on the main stem of arrowleaf clover. The time to axillary leaf appearance was affected (P<0.018) by a species by sowing date interaction. For example, in gland clover, the Tt requirement from sowing to axillary leaf appearance was 642 (±49.1) °C d when sown on 30 March, 2010 (SD2), but it only took 320 (±15.6) °C d (which is half of the Tt for SD2) to produce the first axillary leaf when seeds were sown on 9 November, 2010 (SD8) (Figure 1c).

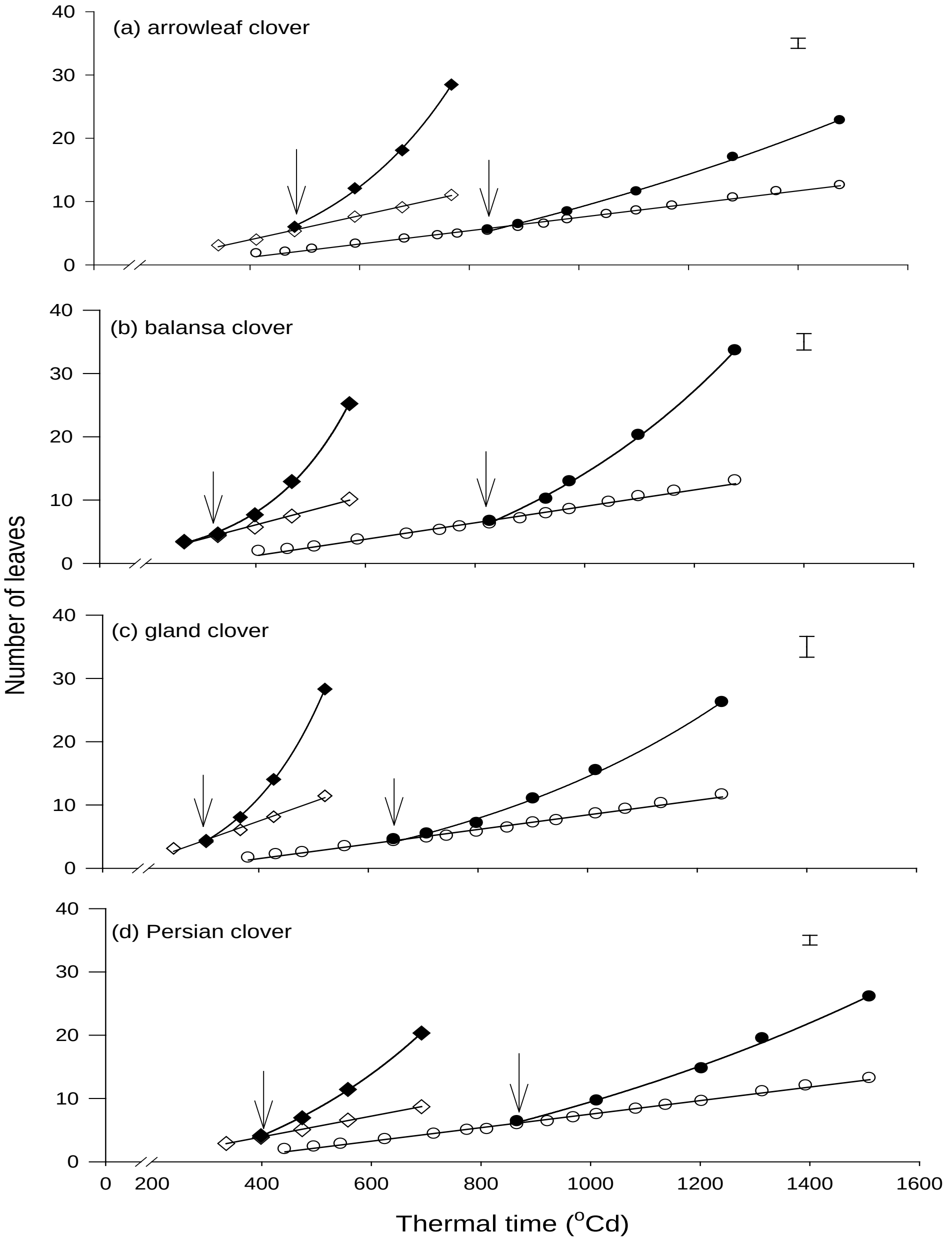

Figure 1. Number of total (closed symbols) and main stem (open symbol) leaves of (a) arrowleaf, (b) balansa, (c) gland and (d) Persian clover plotted against thermal time after sowing (T_b = 0°C, air temperature) of SD2(●) and SD8(◆). Arrows indicate time of axillary leaf appearance. Error bars represent the maximum standard error for the final total leaf number.

The differences in timing of axillary leaf appearance among sowing dates were caused by the differences in the phyllochron at sowing. The phyllochron for gland clover was 87 (±0.5) °C d/leaf in SD2 compared to 33 (±0.1) °C d/leaf in SD8 (Table 1). Thus, the longer phyllochron resulted in a longer duration to the first axillary leaf appearance.

Conclusion

Phyllochron and axillary leaf appearance were quantified by thermal time when photoperiod at emergence was accounted for. Throughout all the sowing dates, phyllochron was fastest for gland (33 – 91°C d) and slowest for arrowleaf (53 – 116 °C d) compared with balansa (44 – 82°C d) and Persian (61 – 93°C d) clovers. To maximize dry matter production and seed yield, autumn sowing is recommended.

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