Leaf development and dry matter production of subterranean clover cultivars in relation to autumn sward management

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Abstract
Seeds of five cultivars of subterranean clover, together with one of white clover, were sown in a wide range of temperature regimes under both controlled environment and field conditions. Results were consistent across temperature regimes and showed that the first trifoliate leaf emerged after 230 °Cd for all subterranean clover cultivars and 309 °Cd for the white clover cultivar. For subterranean clover, exponential leaf appearance commenced after 434 °Cd at the six total leaf stage. At this time, subterranean clover can be defoliated without causing permanent physical damage to seedlings. The field study at Lincoln University showed that subterranean clover that germinated in March produced 44 kg DM/ha/day for 158 days to yield approximately 7 000 kg DM/ha by mid-September. Subterranean clover that germinated in May produced 15 kg DM/ha/day for 120 days to yield only 1 800 kg DM/ha by mid September. These results are discussed in relation to the time of autumn grazing management for subterranean clover, including extrapolation to other climatic areas of New Zealand.

Key words: herbage yield, phyllochron, seedling establishment, thermal time, *Trifolium subterraneum*, *T. repens*, white clover

Introduction
Subterranean clover (*Trifolium subterraneum*) is a winter annual legume adapted to Mediterranean-type climates with warm mild winters and hot dry summers. The ultimate success or failure of the species depends on the number of seedlings that establish each autumn. Estimates of threshold seed reserves in the soil for the success of subterranean clover range from 200–600 kg/ha (Rossiter 1966; Carter & Cochrane 1985; Dear *et al.* 1993). Allowing for the effects of seed dormancy, hardseededness and seed destruction by pests and diseases, Smetham (2003) calculated that the minimum amounts of seed required were 127, 108 and 82 kg/ha for early-, mid- and late-flowering cultivars, respectively.

In areas of New Zealand where subterranean clover grows, the timing of the autumn rains required for germination is extremely variable, and may range from January to May. Following germination, it is generally accepted that subterranean clover pastures should be spelled until the seedlings are sufficiently well developed to survive grazing. Thomas (2003) indicated that the growing points of subterranean clover are pulled closer to the soil surface as the seedlings develop, conferring protection from grazing. However, no consistent criteria are available to define when the first grazing should occur in different sites or seasons.

As temperatures decline markedly between January and May, it is likely that the chronological time required (in days) for seedlings to reach a critical size will increase accordingly. However, as the chronological time requirement varies with season, its practical use in assisting decisions on grazing management is limited. Black *et al.* (2002) showed that, providing moisture is adequate, seedling leaf appearance is driven by the accumulation of thermal time (Tt) or degree-days (ºCd). Specifically, the Tt required for the initiation of branch structures (stolons, runners or tillers) in seedlings has been identified as a key physiological parameter determining the successful establishment of pasture species. This criterion can assist management decisions on time of sowing and compatibility of species within a seed mixture. The same approach could also be used to assist guidelines for autumn grazing of subterranean clover.

Thus, the objective of this research was to quantify the Tt requirements for key development stages (first trifoliate leaf appearance, branch development, phyllochron) of subterranean clover seedlings using a combination of controlled environment and field experiments. The objective of the field experiment was to examine whether the results obtained in controlled environments could be replicated under field conditions. There were five cultivars of subterranean clover examined in this study. White clover (*T. repens*) was also included, both as a reference point (to compare with other studies in the literature), and because it is the most common pasture legume used in New Zealand.

Materials and methods

Controlled environments

A series of six controlled environment experiments was conducted. Each experiment was a randomised complete block design consisting of several clover cultivars with three replicates, but each experiment was conducted at a different nominal temperature (Table 1). For split temperatures, a 12 h/12 h temperature regime was used.

Table 1 Subterranean and white clover cultivars and temperature regimes used for the six controlled environment experiments at Lincoln University.

Cabinet experiment number	Nominal temperature (°C, day/night)	Cultivars[1]
1	6	Demand, Denmark
2	7	Demand, Campeda, Denmark, Goulburn, Leura, Woogenellup
3	18	Demand, Denmark
4	16/8	Demand, Campeda, Denmark, Goulburn, Leura, Woogenellup
5	25/15	Demand, Campeda, Denmark, Goulburn, Leura, Woogenellup
6	25	Demand, Denmark, Campeda, Goulburn, Leura, Woogenellup

[1] 'Demand' is a white clover (*Trifolium repens*). All other cultivars were *T. subterraneum* ssp *subterraneum*.

'Demand' white clover was included in all experiments, while all other clovers were subterranean clover ssp *subterraneum*. Plastic pots 0.37 m in diameter and 0.30 m deep were filled with 3–4 month potting mix. Each plot was designated as 358 cm^2, one third of the total area of the pot, and sown with 50 viable inoculated seeds/plot.

Immediately after sowing, all pots were watered and placed in a controlled environment cabinet at the designated temperature for an 8 h/8 h day/night photoperiod with a 4 h transition period at either end. Light reaching the leaf canopies had a photosynthetic photon flux density of 350 ± 50 µmol/m^2/s and relative humidity was maintained at 65%. Plants were watered as required based on pot weight, and shifted daily to minimise edge effects.

Actual temperatures were monitored by inserting four temperature probes into each of three pots, with one probe (covered with aluminium foil) being placed 10 mm above ground and the other three 10 mm below the soil surface. Such placement has been recommended for accurate prediction of leaf appearance rates in crops (Peacock 1975; Jamieson *et al.* 1995). Temperatures were recorded every 2 minutes and integrated every 30 minutes with a HOBO data logger to determine mean daily temperatures. Due to small variations between nominal temperature settings and the soil surface temperatures the plants experienced, we used the latter for all analyses.

Seedlings were repeatedly thinned before they touched each other to avoid competition. Final populations were 16 plants/plot, and within this population, three plants/plot were marked with coloured wires for detailed monitoring.

Field experiment

A randomised split-plot design was sown in four replicates with 7 March (SD1), 21 March (SD2), 11 April (SD3) and 7 May (SD4) 2003 as the sowing dates for main plots, designed to span the expected range of timing of autumn rainfall. Sub-plot treatments were 'Denmark', 'Campeda', 'Goulburn', 'Leura' and 'Woogenellup' subterranean clovers and 'Demand' white clover sown in 1.0 x 1.0 m plots.

The experiment was conducted in Iversen Field at Lincoln University on a Templeton silt loam soil, which was cultivated to produce a fine, firm seedbed using conventional methods. During cultivation, 200 kg/ha of superphosphate (9% P, 12% S) was incorporated to correct nutrient deficiencies indicated by a soil test.

Inoculated seed was broadcast at a range calculated to sow 2 000 viable seeds/m^2 based on germination tests and thousand seed weights. Seeds were raked in and the experimental area kept moist using a garden sprinkler as required for the first sowing date. Significant rainfall (30 mm) on 28 March 2003 ensured seeds from all other sowing dates were sown into moist soil.

Soil temperature was recorded at 5 mm depth, every 2 minutes, and integrated every 30 minutes with a HOBO data logger to determine daily mean temperatures. All plots were hand weeded when necessary. Three plants in each sub-plot were marked with coloured wires for detailed monitoring.

Measurements

The number of emerged leaves on the monitor plants was counted daily until individual white clover plants had produced five leaves. Leaves were considered emerged as soon as the petiole was visible. The initiation of a stolon or runner in white and subterranean clovers, respectively, which emerge in the axils of main stem (MS) leaves, was also recorded. A stolon or runner was defined as initiated when one leaf had emerged on that axillary bud (Black *et al.* 2002).

Dry matter (DM) production was determined from one 0.2 m^2 quadrat cut to ground level in each sub-plot at approximately monthly intervals. Actual harvest dates were 13 May, 18 June, and 7 August, with the final cut on 11 September 2003. Herbage was dried to constant weight at 70 ºC.

Data analysis

Data for each species were plotted as the reciprocal of the duration (in days) to the appearance of the first trifoliate leaf (MS leaf two) or the initiation of stolon or runner structures against the mean temperature. The inverse of duration (1/days) represents the development rate (Moot *et al.* 2000). A linear relationship indicates that the base temperature and Tt requirements for each development phase can be calculated from the least squares regression coefficients where:

$$\text{Rate} = b_o + b_1 x \qquad \text{Equation 1}$$

The coefficients can then be used to calculate T_b (°C) and Tt (°Cd) as:

$$T_b = -b_o/b_1 \qquad \text{Equation 2}$$

and

$$Tt = 1/b_1 \qquad \text{Equation 3}$$

Phyllochron was calculated as the Tt interval between the appearance of successive MS leaves from the first trifoliate leaf (MS leaf two) to the final MS leaf counted (MS leaf four or five in white and subterranean clovers, respectively).

Results

Leaf appearance

The sequence of leaf appearance was consistent across treatments but differed for white and subterranean clovers (Figure 1). For white clover, the first leaf produced by a stolon was leaf number four, and this was produced at approximately the same time as leaf number five (MS leaf four). However, in subterranean clover, the first leaf produced by a runner was leaf number five, which appeared at approximately the same time as leaf number six (MS leaf five), regardless of cultivar. Notable was that the stolon of 'Demand' white clover was initiated in the axil of the spade leaf (MS leaf one), whereas the runner of subterranean clover was initiated in the axil of the first trifoliate leaf (MS leaf two) for all cultivars.

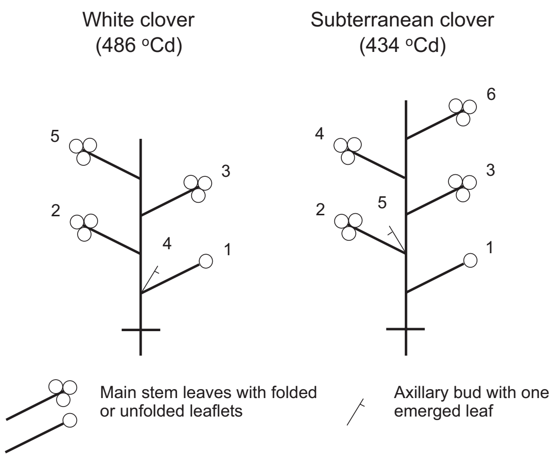

Figure 1 Stylised diagrams illustrating the development of seedlings of white and subterranean clovers after 486 °Cd and 434 °Cd, respectively. During the period of growth, internodes on the main stem and stolons or runners (axillary buds) remain short.

The appearance of the first trifoliate leaf of these legumes followed a similar pattern regardless of species or cultivar. In all cases, the greatest number of days to appearance of the first trifoliate leaf was required at the lowest mean temperature. For example, all of the subterranean clover cultivars—as shown for 'Denmark' in Figure 2a—required less than 40 days to produce their first trifoliate leaf from SD4 when the mean temperature was 7.2 °C. However, for the same sowing date, 'Demand' white clover required 75 days to produce its first trifoliate leaf over a period when it experienced a mean temperature of 5.4 °C (Figure 2a). The number of days to the appearance of the first trifoliate leaf then declined exponentially to approximately 10 days for subterranean and 15 days for white clover as temperature increased to a mean of 23.5 °C. This led to a linear increase ($R^2 > 0.88$) in leaf appearance rate—as shown for 'Denmark' and 'Demand' in Figure 2b—which enabled T_b and Tt to be estimated (Table 2).

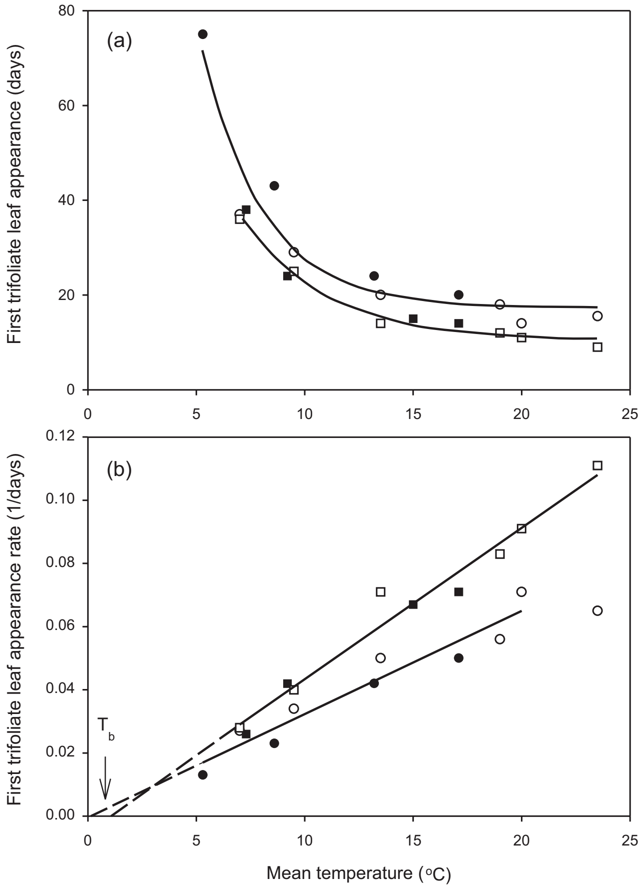

Figure 2 (a) Number of days to the appearance of the first trifoliate leaf and (b) first trifoliate leaf appearance rate of 'Demand' white (●) and 'Denmark' subterranean (■) clovers grown under controlled environment (open symbols) and field (closed symbols) conditions at different temperatures.

To enable direct comparison of the Tt requirements for leaf appearance within and between species, additional regression analyses of rate against temperature were performed with the T_b set at 0 °C (Moot *et al.* 2000). This enabled the Tt requirement (± s.e.) for first trifoliate leaf appearance to be estimated at 230 ± 20.0 °Cd for all subterranean clover cultivars, compared with 309 ± 10.0 °Cd for 'Demand' white clover (Table 2).

The pattern of leaf appearance showed a distinct split at leaf number five and six for white and subterranean clovers, respectively, when the number of MS leaves continued to increase linearly but the total number of leaves increased exponentially. This is shown for 'Demand' and 'Denmark' in Figure 3. The number of days required to reach this point decreased exponentially with increasing temperature, and linear regressions ($R^2 = 0.84$) between stolon or runner initiation rate and temperature enabled T_b and Tt to be estimated (Table 3). Using a common T_b of 0 °C, runners of subterranean clover initially appeared after 434 ± 35.0 °Cd, compared with 486 ± 10.0 °Cd for stolons of white clover. From these data (Tables 2 and 3), the phyllochron (°Cd/leaf) for the MS leaves of subterranean clover was calculated as 68 ± 6.5 °Cd compared with 89 ± 5.4 °Cd for the MS leaves of white clover (Table 3).

Table 2 Base temperature (T_b) and thermal time (Tt) requirements for the appearance of the first trifoliate leaf for 'Demand' white clover and five subterranean clover cultivars grown in controlled environment and field experiments.

Cultivar	T_b (°C)	Tt (°Cd)	R^2 (%)	Tt (°Cd) (T_b = 0 °C)[1]
Demand	0.1	306	91	309
Campeda	1.6	194	96	216
Denmark	1.1	207	97	222
Goulburn	-0.1	231	87	230
Leura	1.0	235	94	251
Woogenellup	0.1	234	97	234

R^2 = coefficient of determination.

[1] Regression analysis with fixed T_b = 0 °C.

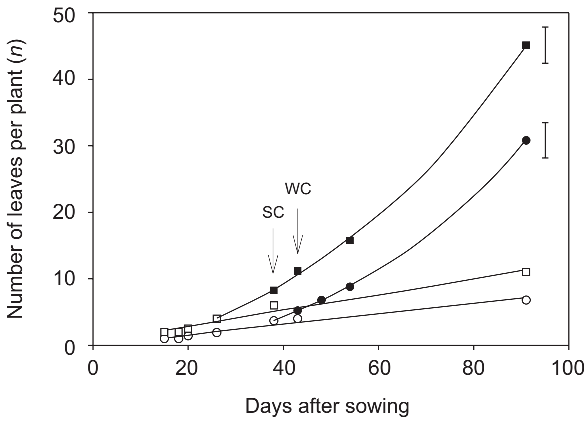

Figure 3 Number of total (closed symbols) and main stem (open symbols) leaves of 'Demand' white (●) and 'Denmark' subterranean (■) clovers plotted against days after sowing in the field on 21 March 2003 at Lincoln University, Canterbury. Arrows indicate the initiation of stolon or runner development (axillary buds) for white (WC) and subterranean (SC) clovers, respectively. Bars represent standard error for final total leaf number.

Table 3 Base temperature (T_b) and thermal time (Tt) requirements for the initiation of stolons (or the five leaf stage) of 'Demand' white clover or runners (or the six leaf stage) of five subterranean clover cultivars grown in controlled environment and field experiments. The phyllochron is the thermal time interval between the appearance of successive leaves on the main stem (MS leaves).

	T_b (°C)	Tt (°Cd)	R^2 (%)	Tt (°Cd) (T_b = 0 °C)[1]	Phyllochron[2] (°Cd/leaf) (T_b = 0 °C)
Demand	-0.8	509	84	486	89
Campeda	-0.7	455	99	434	73
Denmark	-0.2	426	96	422	67
Goulburn	-2.5	504	90	435	68
Leura	-1.3	494	96	456	68
Woogenellup	-0.7	442	95	423	63

R^2 = coefficient of determination.

[1] Regression analysis with fixed T_b = 0 °C.

[2] Phyllochron was calculated from the first trifoliate leaf (MS leaf two) to four or five MS leaves of white and subterranean clovers, respectively.

Dry matter production

'Denmark' and 'Woogenellup' subterranean clovers are used as examples of the effect of sowing date on DM production for the five cultivars grown in the field experiment. By mid-September, subterranean clover that germinated on 7 March (SD1) had produced approximately 7 000 kg DM/ha, while subterranean clover that germinated on 7 May (SD4) had produced only about one quarter of this yield at approximately 1 800 kg DM/ha. Thus, subterranean clover that germinated on 7 March produced 44 kg DM/ha/day for 158 days compared with ($P<0.05$) 15 kg DM/ha/day for 120 days from that which germinated on 7 May.

Discussion

The consistency of the results from both the controlled environment and field experiments showed that thermal time was the main determinant of first trifoliate leaf appearance in subterranean (230 ºCd) and white (309 ºCd) clovers (Table 2). The phyllochron for white clover (Table 2) was similar to that reported by Black *et al.* (2002), who indicated species differences contributed to seedling competitiveness and survival at establishment. The validity of using Tt to quantify seedling development is supported by the consistency of results from controlled environments and the fluctuating diurnal temperature regimes in the field. Thus, the results should be transferable beyond the site or season of investigation. Importantly, the Tt required to reach the start of an exponential increase in the number of leaves per plant was consistent across treatments, which means that this key development stage (Black *et al.* 2002) can be estimated from soil temperature data at any site after the onset of autumn rainfall.

The present study included a simple 'finger and thumb' test to estimate whether the subterranean clover seedlings would tolerate grazing. There was minimal seedling failure at the six-leaf stage, which suggests root and shoot growth were adequate to sustain grazing. However, this test also highlighted several complicating factors. Firstly, the prostrate growth habit of the *subterranean* subspecies made them more tolerant of grazing at juvenile growth stages than the more erect *yanninicum* subspecies. Secondly, where very high (~1 000/m^2) populations of seedlings were established, the plants remained susceptible to grazing past the six leaf stage, as even the *subterraneum* subspecies retained an erect, etiolated growth habit under these conditions. Presumably the low light conditions within the dense sward were responsible. Thirdly, soil type had a marked effect on the ease with which seedlings could be pulled out of the soil by the test. Seedlings grown in loose friable potting mix were much more easily removed at this stage of development than those growing in a silt loam soil in the field. This may have practical implications for the time of grazing in areas such as the coastal sand country in the Manawatu, where subterranean clover is grown on very loose, easily eroded soils composed mainly of sand. However, we concede that these results are preliminary and that experiments should have begun earlier to identify the earliest possible time of success.

Thomas (2003) provides indirect evidence for the likely tolerance to grazing or otherwise of subterranean clover seedlings. He provided three diagrams of developing seedlings. The first seedling, with cotyledons only, and the second, with a total of two leaves, both appear to be susceptible to grazing because the cotyledons are clearly elevated above the soil. At these stages grazing would probably remove all potential growing points. The third diagram shows that by the time four leaves are present the hypocotyl has drawn the base of the cotyledons below soil level, and the axillary buds of the spade and first trifoliate leaf to approximately soil level. In this position, the growing points are unlikely to be removed by grazing unless the whole seedling is pulled out of the ground. On this basis, and considering the reservations discussed previously, the Tt requirement for the development of six leaves (including the spade leaf) presents a simple and repeatable criterion for the timing of first grazing of subterranean clover seedlings.

All of the five subterranean clover cultivars examined in the present study produced six leaves after approximately 434 °Cd from germination (Table 3). In areas of New Zealand where subterranean clover is grown, the timing of autumn rainfall required to allow germination of the seed is extremely variable, and may range from February to May. Table 4 lists the theoretical chronological time to safe grazing of subterranean clover, assuming rain arrives on the first day of each month for a range of sites in New Zealand. In a mild area, like Napier, only 23 days are required following a 1 February rain, but this extends to 39 days for 1 May. In contrast, in cooler areas like Alexandra, following a 1 February rainfall, 26 days are required before grazing can commence, while seeds that germinate on 1 May are vulnerable to frost heave due to their slow growth and development. If they survive, they would require 102 days before reaching the six-leaf stage.

Table 4 Chronological time (days) to safe grazing of subterranean clover at several New Zealand sites with varying dates of opening autumn rains (actual days calculated from NIWA meteorological data summaries, and Lincoln data from Broadfields Meteorological station, using a base temperature of 0 °C).

	Time of Opening Rain			
Location	February 1	March 1	April 1	May 1
Lincoln	26	29	37	53
Alexandra	26	30	46	102
Blenheim	25	27	34	47
Napier	23	25	30	39

The present study also showed that subterranean clover that germinated in March produced substantially more herbage than that which germinated in early May. The cumulative yields were approximately 7 000 and 1 800 kg DM/ha, respectively, by mid-September. Silsbury & Fukai (1977) also found autumn–winter yield was maximised by early germination. Some care must be taken in extrapolating the yields reported in the present study to the practical farming situation. Firstly, the experiment was conducted on a deep, moisture-retaining soil usually used for intensive cropping, and where white clover is the normal pasture legume grown, especially where irrigation is available. On shallow, low fertility soils, yields have been approximately half those reported here (Smetham & Jack 1995; Scott 1971; Ledgard *et al.* 1987). Secondly, the swards comprised of pure subterranean or white clover, and were hand weeded as required. The pros and cons of growing subterranean clover alone or in a mixture with grasses have been well reviewed by Smetham (2003). He concluded that under New Zealand conditions, subterranean clover is usually grown with a native or introduced grass to provide feed longer into summer and help reduce ingress of annual weeds, despite the reported yield advantage of clover monocultures.

Resident grasses are always present in hill country pastures.

Subterranean clover seedlings are sensitive to grass dominance and shading (McGuire 1983). On non-arable country, grass competition can only be reduced by grazing management, but a balance needs to be reached between under- and overgrazing, especially by sheep, which can lead to extensive areas of bare ground and compacted soil (Costello & Costello 2003). Where the paddock can be cultivated, the production of 'top-worked subterranean clover greenfeed' is recommended (Calder 1954). This practice consists of light cultivation with a grubber or discs followed by harrowing and perhaps rolling. Fertiliser may be incorporated into the soil at the same time. When autumn rains arrive, the resultant establishment and growth of subterranean clover can be significant. Top working has the potential to turn a 'false break' into a successful establishment by greatly reducing the competition from other species, all of which can be considered weeds in this situation.

Conclusions

This study provided quantifiable criteria for the timing of first grazing of subterranean clover seedlings. Specific conclusions were:

1. Leaf appearance rate in subterranean clover is determined by Tt, with a thermal time requirement for first trifoliate leaf appearance of 230 ºCd, and a phyllochron of 68 ºCd, measured across five cultivars.
2. Subterranean clover seedlings are likely to survive grazing after they have produced six leaves. The chronological time (days) taken to reach this stage depends on district and timing of autumn rainfall, but is constant in Tt at 434 °Cd.
3. Subterranean clover that germinated on 7 March produced three times the amount of DM than that which germinated on 7 May. This increase in DM production was out of proportion to the chronological time involved, because this process is driven by both thermal time and light interception.

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