Growth and phenological development patterns differ between seedling and regrowth lucerne crops (*Medicago sativa* L.).

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Abstract

This study compared physiological responses of fully-irrigated seedling and regrowth lucerne crops (*Medicago sativa* L.) grown under similar environmental field conditions. Measurements occurred for 2–4 years after sowing on 24 Oct, 15 Nov, 05 Dec and 27 Dec 2000 at Lincoln, Canterbury, New Zealand. Irrespective of the date of sowing, on average lucerne accumulated less shoot dry matter (DM) in the seedling year (11±0.44 t.ha^{-1}) than during the regrowth year (18±0.76 t.ha^{-1}). Slower shoot-growth rates in seedlings were

explained by less intercepted light and reduced efficiency in conversion of light to biomass. Specifically, seedlings had a longer phyllochron ($47\pm2.3°$Cd leaf^{-1}) and slower leaf area expansion rate (0.009 m^2 m^{-2} $°$Cd^{-1}) than regrowth crops ($35\pm1.8°$Cd leaf^{-1} and 0.016 m^2 m^{-2} $°$Cd^{-1}, respectively). There were no differences in canopy architecture with a common extinction coefficient of 0.93. The radiation use efficiency (RUE) for shoot production (RUE$_{shoot}$) was 1.2 ± 0.16 g DM MJ^{-1} of intercepted photosynthetically active radiation (PAR$_i$) in seedlings and 1.9 ± 0.24 g DM MJ^{-1} PAR$_i$ in regrowth crops. Reproductive development was slower in seedling than regrowth crops due to an apparent juvenile period ranging from 240 to 530$°$Cd in seedlings. For both seedling and regrowth phases, the thermal time accumulation to reach 50% buds visible (Tt$_{0-bv}$) and 50% open flowers (Tt$_{0-fl}$) increased as photoperiod shortened in autumn. The minimum Tt$_{0-bv}$, or the thermal-time duration of the basic vegetative period (Tt$_{BVP}$), was estimated at $270\pm48°$Cd at photoperiods >14 h for regrowth crops. The theoretical threshold below which reproductive development is projected to cease, or the base photoperiod (Pp$_{base}$), was estimated at a common 6.9 h for seedling and regrowth crops. The transition from buds visible to open flowers (Tt$_{bv-fl}$) was mainly controlled by air temperature and ranged from 161$°$Cd for seedlings to 274$°$Cd for regrowth crops. These results can be used as guidelines to develop differential management strategies for seedling and regrowth crops and improve the parameterization of lucerne simulation models.

Introduction

The development of sustainable best management strategies for lucerne (*Medicago sativa* L.) production depends on understanding the physiological mechanisms that control yield formation and crop persistence (Fick *et al.*, 1988; Moot and Teixeira, 2005). For the regrowth phase of the crop, the period after first harvest, the responses of lucerne plants to environmental signals and management have been recently quantified experimentally (Brown *et al.*, 2005b; Brown *et al.*, 2006; Teixeira *et al.*, 2007b). These studies have however not provided a direct comparison between seedling and regrowth phases. There is limited information on crop physiological responses during the seedling phase, particularly during reproductive development. Lucerne performance during the seedling phase is a key determinant of plant establishment because it influences crop productivity and stand persistence (Fick *et al.*, 1988). Biomass accumulation during the seedling phase can represent a large proportion of the annual production in farming systems that use lucerne for short periods, such as for inter-cropping (Angus *et al.*, 2000). Improved understanding of physiological responses during the seedling phase may also increase the accuracy of current lucerne simulation models for the prediction of yield and development (Confalonieri and Bechini, 2004; Fick, 1984; Robertson *et al.*, 2002; Teixeira *et al.*, 2009). These models often utilize the analytical framework developed by Monteith (1972; 1994) and this can be used as a basis for comparison between seedling and regrowth phases. In this framework, crop biomass is a linear function of accumulated intercepted photosynthetically active radiation (PAR_i), the slope being the radiation-use efficiency (RUE) for shoot production (RUE_{shoot}). Light interception is modulated by canopy expansion through the rate of leaf appearance and leaf area expansion, which are both mostly driven by temperature (Robertson *et al.*, 2002).

Another important aspect is the quantification of the progression from the vegetative to the reproductive phase in lucerne crops. The time of flowering in lucerne has frequently been used as the basis for formulating agronomic advice in relation to time of defoliation (Fick *et al.*, 1988). From visual observation in the field, the first sign of reproductive development in lucerne is the appearance of floral buds on the seedling crops. In subsequent regrowth crops there is some evidence that the time to flower may be shorter than for seedling crops, but comparative field measurements are rare and model parameterization for lucerne phenology is often derived from controlled environment studies (Pearson and Hunt, 1972a; Robertson *et al.*, 2002). Here we provide a direct comparison between seedling and regrowth lucerne crops grown under similar environmental and field conditions to quantify differences in physiological responses for both vegetative and reproductive phases. Our aim is to enhance the fundamental understanding of the physiology of lucerne seedlings to provide a basis for the development of alternative management strategies and improve lucerne model parameterization.

2. Materials and Methods

2.1 Experimental design and treatments

A field experiment with fully irrigated lucerne 'Grassland Kaituna' was established as a randomised complete block design with three replicates. Plots (4.4 x 10.0 m) were spring sown on (i) 24 October; (ii) 15 November; (iii) 5 December; and (iv) 27 December in 2000 (Table 1). This provided seedling and regrowth lucerne crops at different stages of

development. Plant growth and development were measured in all crops during the first growing season (2000/01). In addition, selected data from the subsequent regrowth cycles of the following 2001/02 growth season, partially presented in Brown *et al.* (2005b), were re-analysed to allow further comparison between seedling and regrowth crops grown under similar temperature and photoperiod conditions. The crop sown on 24 October was continually monitored from 2002 to 2004 for the timing of occurrence of floral buds and flowers to increase the number of data points and the range of environmental conditions in which reproductive development measurements were taken. During the third (2002/03) and fourth (2003/04) seasons of regrowth, the crop was used in a harvest frequency experiment with four replicates (Teixeira *et al.*, 2007b). During these four years of monitoring, irrigation and mineral fertilizer were applied to ensure crop growth and development were unconstrained. Detailed descriptions of site management, experimental design and additional measurements for each experiment were published in Teixeira *et al.* (2007b) and Brown *et al.* (2005b).

2.2 Site characteristics, meteorological conditions and crop management

The experiment was held at Lincoln University, Canterbury, New Zealand (43° 38 'S, 172° 28 'E, 11 m a.s.l.). The soil is a Wakanui silt loam (Udic Ustochrept, USDA Soil Taxonomy) with 1.8–3.5 m of fine textured material overlying gravels. Soil fertility was evaluated from soil samples collected in September 2000 (prior to sowing). Most macronutrients were above recommended levels (Morton and Roberts, 1999). The pH ranged from 6.1 to 6.5; Ca from 9 to 10 meq.100g^{-1}; K from 12 to 18 meq.100g^{-1}; P from 17 to 20 me.100g^{-1}; Na from 8 to 13 me.100g^{-1}. The S concentration in September 2000 (prior to experiment establishment) was

low at 6 ppm. Therefore, 250 kg.ha^{-1} of sulphur super phosphate was applied and the S concentration was then maintained above 10 ppm.

The climate is characterised by evenly distributed annual rainfall of about 640 mm, with slightly higher monthly totals in winter. The annual mean temperature is 11.4°C varying from a monthly average of 6.4°C in June to 16.6°C in January. The range in the average daily total solar radiation was from 4.2 MJ.m^{-2}.day^{-1} in June 2002 to 25.4 MJ.m^{-2}day^{-1} in December 2000. Daily meteorological data used in the analysis were measured at Broadfields Meteorological Station (NIWA, National Institute of Water and Atmospheric Research, New Zealand), which is located 2 km north of the experimental site.

The experimental site previously contained a rape (*Brassica napus* subsp. olifera) experiment in the 1999/2000 season. In April 2000 the area was ploughed and sown into oats. From September to October 2000 the paddock was ploughed, roto-crumbled, harrowed and rolled before sowing. The first sowing date treatment and the remaining guard areas were sown on 24 October 2000 using an Øyjoord cone seeder. Other sowing treatments followed on 15 November; 5 December and 27 December 2000 (Table 1). Inoculated 'Grasslands Kaituna' lucerne seeds were sown to 20 mm depth at a rate of 10 kg.ha^{-1} of coated seed with 93% germination. After sowing, the paddock was chain harrowed to ensure seed coverage. Chemical control of weeds and hand weeding were used to reduce competition with the establishing lucerne crops (Brown *et al.*, 2006).

[Table 1 suggested place]

From 24 October 2000 to 12 June 2002, the crops were grazed by sheep of mixed classes after regrowth intervals of at least 30 days (Table 1) to ensure optimum establishment of root reserves and to maximise production (Moot *et al.*, 2003). The grazing duration of the crops ranged from 1 to 7 days depending on biomass availability. Any herbage remaining post-grazing was mechanically mown above crown height to homogenize residual biomass without damaging new growing points.

2.3 Measurements

Shoot biomass accumulation

Shoot dry matter (DM) measurements were taken at 7–10 day intervals, including a cut on the day of grazing, throughout the entire 2000/01 and 2001/02 growing seasons. Above-ground DM was measured by cutting a single 0.2 m quadrat above crown height (to avoid damaging growing points) with a set of hand shears. All shoot samples were dried in a forced air oven at 65–70°C to constant weight.

Appearance of primary leaves

The appearance of primary leaves was quantified on a group of 5–10 marked main stems per plot. The numbers of primary leaves were counted on each marked main stem at 7–10 day intervals starting from seedling emergence, or the beginning of regrowth, until the onset of flowering. Only 'dominant main stems' were marked, namely the tallest one third of the shoot population, because these account for the majority (>80%) of the shoot yield in lucerne crops (Teixeira *et al.*, 2007a).

Canopy development and light interception

Daily values of fractional light interception (PAR_i/PAR_o) were estimated for seedling and regrowth crops from linearly interpolated values of diffuse non-interceptance (DIFN) measured using a canopy analyser LAI-2000 (LI-COR Inc., Lincoln, Nebraska, USA). Readings of DIFN were taken in predominantly diffuse light conditions at about 7-day intervals, starting 10 days after the last grazing day of the previous regrowth cycle. For seedling crops, the first measurement was taken 30–60 days after sowing, when canopy height was above the sensor height (~30 mm) and shoot biomass was still negligible. The equipment was set to take one reading above and five readings below the canopy in each plot. The DIFN quantifies the fraction of sky that is not blocked by foliage (Jonckheere *et al.*, 2004) and corresponds to [$1-(PAR_i/PAR_o)$]. The leaf area index (LAI) was estimated from calibrated computations of plant area index (PAI) taken with the canopy analyser simultaneously with DIFN readings. All readings were adjusted for sensor height and converted to LAI using the methodology described in Brown *et al.* (2005b).

Reproductive development

Data on reproductive development were available from the first two growth seasons (2000/2001 and 2001/2002) and from an additional two growth seasons (2002/03 and 2003/2004) when the crop was subjected to a harvest frequency experiment (Section 2.1). The presence of visible buds and flowers was recorded at 7–10 day intervals on the same marked stems used for leaf counting. Observations on marked stems were taken from seedling emergence, or immediately after harvest for regrowth crops, until the onset of flowering. Marked stems that showed no sign of reproductive development by the end of the regrowth interval were protected from animals during the grazing period (~2-6 days) by stock exclusion cages (1.5 m x 1.5 m x 1.5 m) which enabled them to be left intact for measurement in subsequent rotations, along with new shoots in the grazed area, until the occurrence of flowering. Reproductive development was recorded as the date when 50% of marked stems had visible buds and then open flowers. Thermal time requirements (Section 2.4) to reach the 50% buds visible (Tt_{0-bv}) and 50% open flowers (Tt_{0-fl}) stages were calculated from time of emergence (seedling crops), or after harvest (regrowth crops). Consequently, the thermal time requirement from 50% buds visible to 50% open flowers (Tt_{bv-fl}) was calculated by subtracting Tt_{0-fl} from Tt_{0-bv}. The thermal time accumulation and the photoperiod (Pp; hours) at the beginning of each rotation were tested as predictors of Tt_{0-bv} and Tt_{0-fl}.

2.4 Calculations

Thermal time accumulation

Daily thermal time (Tt, °Cd) was calculated using a broken-stick threshold model where Tt is assumed zero for mean air temperatures (T_{mean}) below the base temperatures (T_b) of 1.0°C (Jones *et al.*, 1986; Moot *et al.*, 2001). In this framework Tt is accumulated linearly from T_b until 15°C at a rate of 0.7°Cd °C^{-1} and then at a rate of 1.0°Cd °C^{-1} until the optimum temperature (T_{opt}) of 30°C, which was never exceeded in this study. Thermal time accumulation was then calculated as the sum of daily Tt throughout each regrowth cycle.

Rate of leaf appearance and phyllochron

The phyllochron (°Cd main-stem node^{-1}) was calculated as the linear slope between the number of primary leaves on marked stems and thermal time accumulation from emergence date in seedling crops or from grazing date in regrowth crops.

Extinction coefficient

The extinction coefficient (k) was used as an indicator of canopy architecture. The k was calculated as the linear slope between the natural log of diffuse PAR transmission and LAI (Monsi and Saeki, 2005). To ensure independent procedures, light transmission for the k

calculation was measured using a ceptometer (Delta-T devices LTD, 128 Low Road, Burwell, Cambridge CB5 0EJ, England) and plotted against LAI measurements from the canopy analyser.

Leaf area expansion rate

The average leaf area expansion rate (LAER, m^2 leaf m^{-2} soil $°Cd^{-1}$) for a given regrowth cycle was calculated as the linear slope between LAI and thermal time accumulation. Only data points between 5 and 95% of maximum LAI were used. This procedure ensured that periods of senescence that limit LAER, such as flowering or frost, were excluded from the analysis.

Radiation use efficiency for shoot production

The RUE for shoot dry matter production (RUE_{shoot}, g DM MJ PAR_i^{-1}) was calculated from the linear slope of the regression between accumulated shoot DM and accumulated PAR_i within each regrowth cycle (Teixeira *et al.*, 2008). Daily PAR_i was obtained by multiplying the available above canopy PAR for each day (PAR_o) by the fractional PAR interception for each treatment plot. Following the same rationale as for LAI, only data points in the linear phase of biomass accumulation, assumed to be between 5 and 95% of maximum shoot DM, were considered. For calculation purposes, the RUE for total biomass (RUE_{total}, shoots+crowns+roots) was assumed to increase linearly from nil at 0°C to a maximum of

3.16 g DM MJ PAR_i^{-1} at temperatures >18°C. The RUE_{shoot} values were also normalized by T_{mean} during each regrowth cycle to account for temperature effects on net photosynthesis. Possible temperature effects on shoot/root partitioning (e.g. Teixeira *et al.*, 2009) were not considered in the analysis because root biomass was not measured in this study.

Reproductive development

Reproductive development was analysed by adapting the concepts proposed by Major (1980) and Robertson *et al.* (2002). It is assumed that lucerne plants have a basic vegetative phase (BVP), characterized as the minimum thermal time requirement (Tt_{BVP}, °Cd) for the transition from vegetative to reproductive status. The first field observable sign of transition to the reproductive phase is the appearance of visible floral buds, defined as the "buds visible" stage. For regrowth crops, the Tt_{BVP} was estimated as the horizontal asymptote of the exponential fit between Tt_{0-bv} and photoperiod. This model was then simplified to a bi-phasic linear model, consistent with Major *et al.* (1991), where the inflection point is the critical photoperiod (Pp_{crit}). The Pp_{crit} was calculated as the photoperiod when Tt_{0-bv} departed from the 95% confidence interval of the horizontal asymptote for Tt_{BVP}. For long-day plants such as lucerne, Tt_{0-bv} increases quasi-linearly at $Pp<Pp_{crit}$ at a slope defined as the photoperiod sensitivity (Pp_s, °Cd/h). To characterize the theoretical photoperiod when reproductive development ceases, the base photoperiod (Pp_{base}) was calculated by extrapolating the rate of reproductive development to nil. The Tt_{0-bv} difference between seedlings and regrowth crops was assumed as the juvenile period (Tt_{juv}) required for seedlings to reach buds visible. A pooled value for Tt_{juv} was calculated by estimating the best fit, *i.e.* lowest root mean squared

deviation (RMSD), between the Tt_{0-bv} model for regrowth crops and the measured Tt_{0-bv} values for seedling crops. We further compared our results with the controlled environment data from Major *et al.* (1991) and Pearson and Hunt (1972a) by converting their figures from "days to flowering" to "thermal time to flowering" units and then recalculating our results from Tt_{0-bv} to Tt_{0-fl}. Values presented graphically by Major *et al.* (1991) were digitized using data extraction software GetData (www.getdata-graph-digitizer.com).

2.5   Statistical analysis

Statistical analyses were performed using GenStat 11th edition (VSN International). When necessary, analysis of variance (ANOVA) was used to partition the observed variation between treatment effects and errors. Fisher's least significant difference (LSD) was used to ascertain the extent of difference between different levels of a factor when the ANOVA gave a P-value of 0.05. To search for explanatory relationships, yield-forming variables were regressed against a crop or environmental continuous variable. All regression analyses were carried out with a model/loss fitting procedure using Sigmaplot 10.0.1.2 (Systat Software, Inc).

3. Results

3.1 Shoot dry matter yield

In the establishment season of 2000/01, the first crop sown on 24 Oct yielded 14.5±0.86 t.ha^{-1}, or 30–40% more (P<0.01) than the latest sown crops (Figure 1a).

[Figure 1 – suggested place]

In the second 2001/02 season, regrowth crops yielded up to 20±1.2 t.ha^{-1} (Figure 1b). Shoot yield was similar for all three initial sowing dates. The only difference was observed for the latest sowing on 27 December, which was 15% less productive (P<0.001) than the other crops.

3.2 Primary leaf appearance rate

Lucerne crops expanded up to 18 leaves during the seedling and regrowth phases (Figure 2). The rate of primary leaf appearance ranged from 0.018 leaves per day for the seedling crop sown on 5 December, to a maximum of 0.031 leaves per day in the summer regrowth cycles.

[Figure 2 – suggested place]

The phyllochron in seedling crops was 47°Cd per primary leaf, or 34% longer (P<0.001) than the 35°Cd calculated for regrowth crops (Table 2).

3.3 LAI and leaf area expansion rate

Final crop LAI ranged from 3.5 to 5.5 throughout the growing seasons (Figure 3). Leaf area expansion rates (LAER), the slope between LAI and thermal time accumulation differed between seedling and regrowth phases. The LAER was 0.009 $m^2.m^{-2}.°Cd^{-1}$ in seedling crops, or 45% lower (P<0.01) than the 0.015 $m^2.m^{-2}.°Cd^{-1}$ calculated for regrowth crops (Table 2).

[Figure 3 – suggested place]

3.4 Light interception

Seedling and regrowth crops showed a similar (P<0.13) pattern of increasing fractional light interception with leaf area index (Figure 4). Both crops achieved 95% light interception, the critical leaf area index (LAI_{crit}), at an LAI of 3.6. As a consequence, the calculated extinction coefficient for incoming PAR (k) for both seedling and regrowth crops was 0.93.

 [Figure 4 – suggested place]

3.5 Radiation use efficiency for shoot production

The highest crop yields for individual regrowth cycles were ~500 g $DM.m^{-2}$ or 5.0 t $DM.ha^{-1}$ (Figure 5). To achieve this yield, seedling crops required the interception of ~400 MJ PAR_i m^{-2} compared with ~300 MJ PAR_i/m^2 for regrowth crops.

[Figure 5 – suggested place]

Therefore, the RUE_{shoot} for seedling crops was 1.2±0.16 or 34% lower (P<0.001) than the 1.9±0.24 g DM.MJ PAR_i^{-1} for regrowth crops (Table 2). After a normalization for temperature during each regrowth cycle (Brown *et al.*, 2006), the difference in RUE_{shoot} between seedling and regrowth crops remained at 40%.

[Table 2 – suggested place]

3.6 Reproductive development

Time to reach 50% buds visible

Irrespective of time of the year, seedling crops consistently required longer periods (P<0.01) to reach the 50% buds visible stage than regrowth crops (Figure 6). In the summer of 2000, seedling crops required 44 days to reach 50% buds visible compared with 27 days in regrowth crops. In both crops, the time to reach 50% buds visible was less in summer than in winter. For example, seedling crops that emerged in early June required nearly 200 days to reach 50% buds visible while only 56 days were needed when emergence was in November.

[Figure 6 – suggested place]

Thermal time to reach 50% buds visible stage

16

Seedling crops consistently showed higher values of Tt_{0-bv} than regrowth crops throughout the entire photoperiod range (Figure 7). In seedlings, the Tt_{0-bv} at the shortest photoperiod (10 h) was 1200°Cd but declined (P<0.01) linearly to a minimum of 500°Cd at the longest photoperiod of 16.5 h. For regrowth crops, the Tt_{0-bv} declined (P<0.01) from 700°Cd at a 10 h photoperiod to a projected minimum of 270°Cd beyond 14 h, the inflection point in the linear bi-phasic model (R^2=0.84). The additional Tt_{0-bv} required by seedlings to reach buds visible, or the juvenile period (Tt_{juv}), ranged from a minimum of 330±32°Cd at Pp>14h to 530±0.50°Cd below that (Figure 7).

[Figure 7 – suggested place]

The rate of change of Tt_{0-bv} with Pp below Pp_{crit}, named the photoperiod sensitivity (Pp_s), was -106°Cd/h for both seedling and regrowth crops.

Thermal time to reach 50% open flowers stage

After the buds visible stage, temperature was the main driver of development. This was shown by the linear relationship (P<0.0001) between Tt_{0-bv} and Tt_{0-fl} in which there was no evidence (P<0.01) that the slope was different from 1.0 (Figure 8). The thermal time requirement from buds visible to open flowers (Tt_{bv-fl}), *i.e.* the *y*-intercept, was 161°Cd for seedling crops and 274°Cd for regrowth crops.

[Figure 8 – suggested place]

Base photoperiod

The base photoperiod (Pp_{base}), the theoretical photoperiod when development ceases, was estimated in common as 6.9 h for seedling and regrowth crops (Figure 9). This was calculated as the *x*-intercept extrapolated from the linear relationships between photoperiod below the Pp_{crit} of 14 h, and the rate of bud appearance on a thermal time basis. For this calculation, the only three available Tt_{0-bv} estimates in seedling crops were subtracted from the $530^{\circ}Cd$ for the juvenile period (Tt_{juv}), and then included in the analysis.

[Figure 9 – suggested place]

Discussion

Growth and development patterns of lucerne differed between seedling and regrowth crops. Seedling crops of lucerne produced less biomass and required longer periods to reach flowering than regrowth crops when grown in a similar environment. Yield differences were explained by different patterns of light interception and conversion of light into shoot biomass.

Light interception

Seedling crops intercepted less light than regrowth crops because leaf area expansion rate (LAER) was 40% slower during the seedling phase than the equivalent regrowth phase (Figure 2). The rate of canopy expansion was previously shown to be a key aspect explaining seasonal yields in regrowth crops (Teixeira *et al.*, 2007c). This reduction in LAER for seedlings was partially explained by a 34% longer phyllochron (Table 2) with consequent reduction in leaf appearance rates. This pattern agrees with re-analysed data for cultivars 'Moapa' and 'Vernal' grown under controlled environments (Pearson and Hunt, 1972a) in which the phyllochron of seedlings was up to 40% longer than for regrowth crops. In the present study, the phyllochron of regrowth crops was 35°Cd, a value which is consistent for diverse cultivars and growth conditions (Brown *et al.*, 2005b; Pearson and Hunt, 1972a; Robertson *et al.*, 2002; Teixeira *et al.*, 2007c). The longer phyllochron during the seedling phase indicates that primary leaf appearance was controlled by factors other than temperature. One possible explanation is that the supply of carbon to shoots is insufficient to meet the potential demand for leaf appearance rates in seedlings, because the formation of roots imposes a strong competing carbon sink. The same rationale may be valid for the limited nitrogen availability caused by plants forming the root system but lacking sufficient rhizobia nodulation to meet nitrogen demand during the seedling phase. This mechanism was suggested by Brown *et al.* (2005b) to explain the higher phyllochron observed during autumn when regrowth lucerne preferentially allocates assimilates to replenish carbon and nitrogen reserves in roots (Avice *et al.*, 2003). Development processes, such as leaf appearance, are however expected to have low sensitivity to carbon supply (Hodges, 1991) which would suggest a severe deficit of carbon during the seedling phase. Alternatively, leaf appearance rates could be lower in seedlings due to an ontogenic-related decline in the plastochron as plant development progresses or due to a lower sensitivity to environmental stimuli imposed by the morphogenetic programme *per se* during this phase. These hypotheses could also

explain the additional thermal time requirement for seedlings to reach 50% buds visible, the juvenile period (Figure 7). The longer phyllochron in seedlings was only partially responsible for the slower LAER, that was reduced twice as much as leaf appearance (Table 2). The implication is that the expansion of individual leaves (not measured) was also reduced in seedling crops. The relevance of individual leaf expansion as a driver of LAI development is consistent with previous observations in nitrogen-limited regrowth crops (Teixeira *et al.*, 2007c).

No differences in canopy architecture were found between seedlings and regrowth crops (Figure 4). Canopy architecture, quantified by the value of the extinction coefficient (k) has previously been shown to vary little among contrasting defoliation regimes, shade and water treatments (Teixeira *et al.*, 2007c; Varella, 2002). The calculated LAI_{crit} of 3.6 was slightly higher than the value of 3.2 observed in these previous studies possibly due to differences in the instruments used and destructive measurements taken to measure transmitted light through the canopy (Section 2.4).

Conversion of light into shoot biomass

The pooled RUE_{shoot} during the seedling phase was 40% lower than for the regrowth stage (Table 2). This apparent reduction in conversion efficiency has previously been attributed to a relatively higher allocation of biomass into the root system (Khaiti and Lemaire, 1992). These authors estimated that ~65% of total assimilated carbon is partitioned to roots during the seedling phase compared with ~10 to 50% during regrowth, depending on the time of the year (Khaiti and Lemaire, 1992; Teixeira *et al.*, 2008). Although root biomass was not sampled in the present study, it is possible to estimate biomass partitioning to roots (p_{root}) by

assuming the optimum RUE for total biomass production (RUE_{total}) as a constant (Khaiti and Lemaire, 1992) further adjusted by mean air temperature (Brown *et al.*, 2006). The partitioning to roots can then be calculated as the quotient between RUE_{shoot} and RUE_{total}. In our study, this exercise yielded a pooled p_{root} of $57\pm0.7\%$ for seedlings and $28\pm1.2\%$ for regrowth crops (data not shown). The relatively lower variability in p_{root} for seedlings suggests that environmental factors had less influence on biomass partitioning during this period. This contrasts with the strong seasonal response of partitioning of biomass to roots observed during the regrowth phase (Teixeira *et al.*, 2008). As an alternative hypothesis, the limited RUE_{shoot} in seedlings could be due to a lower leaf photosynthetic capacity during this stage. Limited nitrogen supply to leaves was shown to reduce light-saturated leaf photosynthesis in regrowth crops subjected to frequent defoliations (Teixeira *et al.*, 2008). In the seedling phase, nitrogen supply to leaves could be limited by the absence of root reserve pools (Avice *et al.*, 2003) or nitrate (NO_3^-) availability from the still forming *Rhizobium meliloti* root nodules (Baysdorfer and Bassham, 1985; Caetano-Anolles and Gresshoff, 1991) that fix atmospheric nitrogen (Vance *et al.*, 1979). This initial period of low RUE_{shoot}, further indicated by the projection of negative y-intercepts in seedlings (Figure 5), overlaps with the juvenile period of development (Figure 7) when seedling crops remain vegetative for longer than regrowth crops.

Reproductive development

The patterns of reproductive development also differed between seedling and regrowth lucerne crops. When grown under similar photoperiod and temperature conditions, seedling crops consistently required longer to reach reproductive status than regrowth crops. This

additional temperature requirement was previously observed under controlled conditions by Pearson and Hunt (1972b) and defined as the 'juvenile' period of seedling development.

The rate of reproductive development was consistently slower at shorter photoperiods, regardless of the direction of change in photoperiod, for both seedling and regrowth crops. These field results confirm lucerne as a long-day plant, as previously stated from controlled environment trials (Major *et al.*, 1991). Long-day plants delay flowering when experiencing day lengths shorter than the Pp_{crit}. A proposed explanatory mechanism is that the activity of genes that reduce sink priority of reproductive organs is triggered at short photoperiods (Wallace *et al.*, 1993). Similar seasonal differences in the metabolic activity of lucerne shoots were identified for dry matter partitioning (Avice *et al.*, 2001; Teixeira *et al.*, 2007b) and canopy expansion (Brown *et al.*, 2005b). At photoperiods shorter than the Pp_{crit} of 14 h, Tt_{0-bv} increased at a photoperiod sensitivity rate (P_s) of $106°Cd\ h^{-1}$ for both crops. This is similar to the overall figure of $126±20°Cd\ h^{-1}$ recalculated from Major *et al.* (1991) for different cultivars which indicates some stability of P_s among genotypes. However, in contrast with these authors, the P_{crit} for regrowth crops in our study was 14 h while they observed a Pp_{crit} of ~18 h during the seedling phase. Interestingly, by re-analysing Major *et al.* (1991) and plotting both datasets together, our Tt_{0-fl} model for seedlings intercepts Tt_{BVP} at a similar Pp_{crit} of 18 h (Figure 10). This suggests consistency between seedlings of diverse cultivars and growth conditions and indicates that the juvenile period may be a consequence of the difference in P_{crit} between seedlings and regrowth crops. As a result, Tt_{juv} would become nil at long photoperiods, e.g. Pp_{crit} >18 h in Figure 10.

[Figure 10 – suggested place]

The joint analysis in Figure 10 indicates a consistent minimum requirement of 530°Cd to reach flowering (Tt_{0-fl}) for both seedlings and regrowth crops. This delimits a common basic vegetative period (Tt_{BVP}) for flowering in a diverse group of lucerne cultivars. Given a phyllochron of 47°Cd in seedlings and 35°Cd in regrowth crops (Table 2) this would equate to a minimum of ~11 and 15 leaves before floral development respectively. At photoperiods shorter than 18 h, seedlings increasingly require more Tt to reach flowering. For example, at a Pp of 15.5 h, ~17 leaves would appear in seedlings in comparison to the minimum of 15 in regrowth crops. These values closely compare with the range observed in lucerne 'Moapa' and 'Vernal' grown under controlled conditions (Pp = 15.5 h) at 17–21 leaves for seedlings and 13–14 leaves for regrowth crops (Pearson and Hunt, 1972b).

Photoperiod had no influence on the transition from bud-visible to the flowering stage. Once floral buds were initiated, the rate of development towards a fully opened flower was regulated by temperature. It is unclear why the thermal time accumulation from buds visible to flowering (Tt_{bv-fl}) was 110°Cd higher in regrowth than in seedling crops. It would be expected that once floral buds are formed, metabolic processes controlling the progress towards flowering would impose a similar rate of development regardless of crop stage. Both seedling and regrowth crops showed a common base photoperiod (Pp_{base}) at 6.9 h (Figure 4). This is the theoretical photoperiod threshold at which the rate of progression towards reproductive stage is negligible. The re-analysis of Major *et al.* (1991) data showed a Pp_{base} range from 7.2 to 8.7 h with the exception of cultivar 'Anik' with extreme values of 14.4 h (Figure 10).

This indicates that a wide genetic variability may exist for some, but not all, parameters that characterize reproductive development in lucerne. The sensitivity of physiological processes

to environmental stimuli may differ among lucerne cultivars (Irwin *et al.*, 2001), as observed for winter dormancy of different genotypes. In contrast, some parameters such as the photoperiod sensitivity (P_s) and the thermal-time duration of the basic vegetative period (Tt_{BVP}) were consistent when compared with previously published data from a wide range of cultivars. Other key parameters that influence vegetative growth, such as radiation use efficiency (RUE) and the leaf area expansion rate (LAER), were also consistent among cultivars grown in New Zealand (Teixeira *et al.*, 2008; Teixeira *et al.*, 2007c) and France (Gosse *et al.*, 1984; Gosse *et al.*, 1982) during the regrowth phase.

Conclusions

Seedling and regrowth lucerne crops showed different patterns of growth and development when subjected to similar environmental conditions. Seedling crops consistently accumulated less biomass into shoots and required longer thermal time accumulation to reach flowering. Therefore, environmental factors seem to have had a less pronounced influence on physiological processes during the seedling-phase than the regrowth-phase. The lower yield in seedlings was mainly explained by reduced light interception and low efficiency of light conversion into shoot biomass. The rate of reproductive development was delayed at short photoperiods in both seedling and regrowth crops. From a management perspective, the delay in shoot biomass accumulation in seedlings means harvest will be later at this phase than for corresponding regrowth crops. The delayed canopy expansion is also likely to make the seedling crop more vulnerable to weed competition for light than regrowth crops, which reinforces the need for appropriate pre- and post-emergent weed control in establishing crops. The relationships and parameters found for lucerne in our study could be incorporated into

simulation models to improve the predictions of plant growth, vegetative development and canopy expansion during the seedling and regrowth phases.

Acknowledgments

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Figure captions

Figure 1. Annual shoot dry matter accumulation of 'Grasslands Kaituna' lucerne during seedling year and (b) the following regrowth year for crops sown on four different dates at Lincoln University, New Zealand. Bars represent the standard error of means.

Figure 2. The number (*n*) of primary leaves per main stem against thermal time accumulation (Tt_b=0°C) after emergence for seedling (grey symbols) and regrowth 'Grasslands Kaituna' lucerne crops sown on 24 Oct 2000 (◄), 15 Oct 2000 (▲), 05 Dec (■) and 27 Dec 2000 (♦) at Lincoln University, New Zealand.

Figure 3. Leaf area index against thermal time accumulation (Tt_b=0°C) after emergence for seedling (grey symbols) and regrowth (white circles) 'Grasslands Kaituna' lucerne crops sown on 24 Oct 2000 (◄), 15 Oct 2000 (▲), 05 Dec (■) and 27 Dec 2000 (♦) at Lincoln University, New Zealand.

Figure 4. Fractional interception of photosynethically active radiation against leaf area index for 'Grasslands Kaituna' lucerne crops at seedling and regrowth stages grown at Lincoln University, New Zealand.

Figure 5. Shoot biomass in relation to the intercepted photosynthetically active radiation (PAR_i) accumulated after emergence for seedling crops (grey symbols) or after grazing (white circles) 'Grasslands Kaituna' lucerne crops sown on 24 Oct 2000 (◄), 15 Oct 2000

(▲), 05 Dec (■) and 27 Dec 2000 (♦) at Lincoln University, New Zealand. Regression coefficients for RUE_{shoot} are given in Table 2.

Figure 6. Number (*n*) of days to reach the bud-visible stage for seedling and regrowth 'Grasslands Kaituna' lucerne crops grown at Lincoln University, New Zealand.

Figure 7. Thermal time (Tt_b=0°C) requirement for 50% appearance of buds (Tt_{0-bv}) for seedling and regrowth 'Grasslands Kaituna' lucerne crops grown during a common range of photoperiods at Lincoln University, New Zealand. The dashed line model (for seedlings) is y=2296-106.8x; R^2=0.93. The solid line bi-linear model (R^2=0.84) for regrowth crops is y=-91.29x+1591.2 at Pp<14h and y= 269.00 at Pp≥14 h.

Figure 8. The thermal time requirement for 50% buds visible (Tt_{0-bv}) in relation to 50% flowering (Tt_{0-fl}) for seedling and regrowth 'Grasslands Kaituna' lucerne crops grown at Lincoln University, Canterbury, New Zealand. Solid line model (regrowth) is y=0.99x+274, R^2=0.89, P<0.001 and dashed line model (seedlings) is y=1.01x+161, R^2=0.99, P<0.001.

Figure 9. The rate of bud appearance (1/°Cd) at photoperiods <14 h in relation to photoperiod for seedling and regrowth 'Grasslands Kaituna' lucerne crops grown at Lincoln University, New Zealand. Note: The 14 h is the assumed critical photoperiod (see Figure 7).

Figure 10. Thermal time requirement for lucerne flowering (Tt_{0-fl}) in relation to photoperiod comparing current results with re-analysis of nine different lucerne cultivars. Adapted from Major *et al.* (1991). Note: The Tt_{0-fl} values for the re-analysed dataset were obtained by

converting values from "days" at 25°C in a growth chamber to thermal time assuming accumulation of 20°Cd/day (Section 2.4).

Tables

Table 1. Grazing dates and intervals for lucerne crops grown at Lincoln University, Canterbury, New Zealand from 24 October 2000 to 12 June 2002.

Growth season	Sowing date	Regrowth cycle	Start date	Defoliation Date	Regrowth duration (days)	Grazing duration (days)
Season	2000/2001					
	24 Oct 00					
		1*	24-Oct-00	24-Jan-01	92*	-
	1	2	25-Jan-01	7-Mar-01	41	-
		3	8-Mar-01	30-Apr-01	53	2
		4	2-May-01	4-Jul-01	63	2
	15 Nov 00					
		1*	15-Nov 00	13 Feb 01	90*	-
	2	2	14 Feb 01	30 Apr 01	75	2
		3	1 May 01	4 Jul 01	64	2
	05 Dec 00					
		1*	5 Dec 00	7 Mar 01	92*	-
	3	2	8 Mar 01	30 Apr 01	53	2
		3	1 May 01	4 Jul 01	64	2
	27 Dec 00					
		1*	27 Dec 01	27 Mar 01	90*	-
	4	2	28 Mar 01	30 Apr 01	33	2
		3	1 May 01	4 Jul 01	64	2
	All regrowth treatments**					
Season	2001/02					

1	6-Jul-01	29-Sep-01	85	6
2	5-Oct-01	14-Nov-01	40	6
3	20-Nov-01	21-Dec-01	31	5
4	26-Dec-01	31-Jan-02	36	6
5	6-Feb-02	4-Apr-02	57	5
6	9-Apr-02	12-Jun-02	64	6

Note: *Initial seedling crops. **Includes period for subsequent regrowth cycles, data partially presented in Brown *et al.* (2005a).

Table 2. Calculated physiological parameters for seedling and regrowth 'Grasslands Kaituna' lucerne crops grown at Lincoln University, Canterbury, New Zealand.

Physiological variable	Seedling crops	Regrowth crops
Phyllochron ($°Cd.leaf^{-1}$)*	47±2.3	35±1.8
LAER ($LAI.°Cd^{-1}$)*	0.009±0.0009	0.016±0.016
Extinction coefficient (k)	0.96±0.008	0.89±0.005
RUE_{shoot} (g $DM.MJ PAR_i^{-1}$)*	1.2±0.16	1.9±0.24
Fractional RUE_{shoot} (normalized[a])*	0.6±0.12	1.0±0.15

*Means significantly different at $\alpha=0.05$. [a]RUE_{shoot} normalized by mean air temperature.