

Impact of autumn (fall) dormancy rating on growth and development of seedling lucerne

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Abstract

To quantify the influence of autumn (fall) dormancy (FD) on DM production and phenological development during the seedling phase, three lucerne genotypes with contrasting ratings were grown at Lincoln University, New Zealand. A dormant (FD2), a semi-dormant (FD5), and a winter-active (FD10) genotype were inoculated and sown at a rate of 290 plants/m² on 8 October 2014. By the end of the seedling phase (15 January 2015) the FD10 genotype had produced 20% higher shoot yield and 17% higher root yield than the other two genotypes. The percentage of total biomass partitioned to roots (P_{root}) was 50% for all genotypes. Total plant biomass (root + shoot yields) was 6.55 t DM/ha for FD10 compared with 5.57 t DM/ha for FD 2 and FD5. Plant height at the open-flower stage was 39.5 cm for FD10 compared with 34.5 cm for FD5 and 33.5 cm for FD2. Maximum leaf area index was similar amongst genotypes at 2.5. However, individual leaf area was 142, 119 and 111 cm²/stem for FD10, FD5 and FD2, respectively. The phyllochron was 52°Cd per primary leaf (base temperature of 1°C) and consistent amongst genotypes. The number of primary leaves and branches were also conservative at 17 and 14/shoot, respectively. Therefore, differences in shoot yield among these lucerne genotypes during the seedling stage were mainly due to differences in plant height and individual leaf area expansion per plant. This led to greater light interception and therefore higher total biomass accumulation for FD10 than for the other two genotypes.

Keywords: alfalfa, autumn, *Medicago sativa* L., phyllochron

Introduction

Biomass accumulation can be estimated as the product of radiation intercepted by the canopy and radiation use efficiency. Radiation interception is modulated mainly by canopy expansion during growth. Radiation use efficiency is usually calculated as shoot biomass in relation to the amount of radiation intercepted. These relationships have been determined for many annual crops (Monteith & Moss 1977) and more recently extended to perennial crops (Robertson

et al. 2002; Confalonieri & Bechini 2004) such as lucerne (*Medicago sativa*). They are the basis for crop simulation models and have been integrated into APSIM–Lucerne (Moot *et al.* 2015).

For lucerne, the modelling also has to account for observed seasonal variation in biomass partitioning above and belowground (Teixeira *et al.* 2007b). This framework has been successfully used to simulate shoot yield of Kaituna lucerne, a semi-dormant (FD-5) genotype (Teixeira *et al.* 2009). It is unclear if, or how, these physiological mechanisms might change with genotypes of different fall dormancy (FD). Fall dormancy is the international criterion used to index aboveground shoot height in autumn relative to reference genotypes (Barnes *et al.* 1979). Specifically, non-dormant or winter-active (FD-10) lucerne has been shown to exhibit higher shoot growth rates after defoliation when compared with dormant types (Leach 1969), particularly in autumn. It is unknown whether these dormancy related differences in shoot growth rate are a consequence of contrasts in the partitioning rates of dry matter (C and N) to the crown and taproot. Post-defoliation, a lack of underground reserves can reduce photosynthetic capacity and canopy expansion rates of the earliest initiated leaves (Teixeira *et al.* 2008). Ultimately, this may affect production and persistence of lucerne crops. It is known that genotypes with higher fall dormancy ratings produce more herbage in autumn but are less persistent than those with a lower rating (Harvey *et al.* 2014). To elucidate the physiological mechanisms responsible for these different responses, a systematic investigation of crop growth and development of lucerne stands with different fall dormancy is required. It is also known that the physiological responses of seedling lucerne during taproot establishment, differs from established crops so they must be considered separately (Teixeira *et al.* 2011).

This study describes the initial lucerne growth and development during the seedling phase of three spring sown lucerne crops with different fall dormancy ratings. The objective was to quantify the influence of fall dormancy on DM production and phenological development during the seedling phase, defined as sowing to first harvest.

Materials and methods

Experimental treatments and design

This experiment used three genotypes with contrasting fall dormancy FD: a dormant (FD2), a semi-dormant (FD5), and a winter-active (FD10) genotype. They were established in a randomised complete block design with four replicates. Plots (20 x 4.2 m) were spring sown on 8 October 2014. They were inoculated with NoduleN® and coated lucerne seed of FD2, FD5 and FD10 were sown at 15.1, 11.8 and 11.1 kg/ha, respectively. Rates differed to account for differences in final germination test results with the aim of sowing ~10 kg/ha of bare seed equivalent.

Experimental site, meteorological conditions and crop management

The experiment was located at Iversen Field, Field Research Centre (FRC), Lincoln University. The soil was a Wakanui silt loam (*Aquic Haplustept*, USDA Soil Taxonomy) with a depth of 2 to 3 m consisting of silt to loamy sand. Soil fertility was analysed from a bulk sample of 20 soil cores of the topsoil (0-150 mm) collected randomly from each half (North to South) of the paddock on 3 October 2014 (Table 1). Based on these results no fertiliser was added at establishment.

Soil and air temperature data were collected on-site. A soil temperature (T_{soil}) probe was placed at seed depth about 20 mm below the soil surface, and was monitored from sowing until 50% emergence of the first trifoliolate leaf. The air temperature (T_{air}) was measured by a thermistor installed at 1.5 m above ground. T_{soil} and T_{air} were measured at hourly intervals by a Hobo 4 channel logger and used for thermal time calculations. Other data were collected at the Broadfields Meteorological Station, 2 km north of Lincoln University, which monitors rainfall (mm), Penman potential evaporation (mm), solar radiation ($\text{MJ}/\text{m}^2/\text{day}$), wet and dry bulb temperature ($^{\circ}\text{C}$) and wind run (m/s). The long term average rainfall is 50-60 mm per month but this was only achieved in November (Figure 1). As a consequence, monthly evapotranspiration exceeded rainfall in all months during establishment. Evapotranspiration was

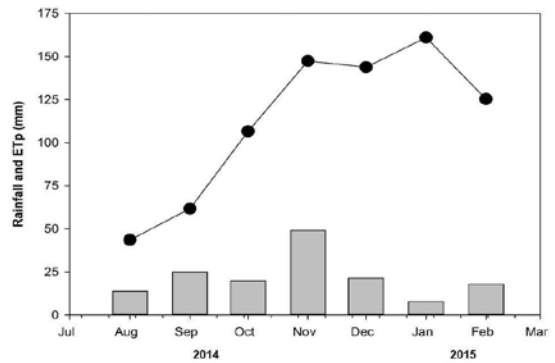


Figure 1 Monthly rainfall (grey bars) and Penman potential evapotranspiration (ETp ●) from 1 August 2014 to 28 January 2015. Data were collected at the Broadfields Meteorological Station, Lincoln.

highest in January 2015 (161 mm) when rainfall was only 8 mm. Vapour pressure deficit (VPD, kPa) ranged from 0.8 kPa in autumn 2014 to 1.5 kPa in summer 2015. Wind run averaged 414 km/day during the experimental period.

Monthly air temperature was highest in January 2015 (17.7 $^{\circ}\text{C}$) with temperature extremes of 30.8 $^{\circ}\text{C}$ on 18 January 2015 and 0.6 $^{\circ}\text{C}$ on 06 November 2014. Monthly values of total solar radiation were highest in December 2014. Daily total solar radiation ranged from 33.7 $\text{MJ}/\text{m}^2/\text{day}$ on 14 December 2014 to 7.4 $\text{MJ}/\text{m}^2/\text{day}$ on 12 January 2015.

Plots were hand-weeded as needed for about 2 months after seedling emergence. On 2 December 2014, all plots were sprayed with Spinnaker (Imazethapyr; 240 g a.i./litre) to control fathen (*Polygonum aviculare*) and other broadleaf weeds. Irrigation water (42 mm, total) was applied on 13 and 14 January 2015 to ensure seedlings established and their growth were not compromised by dry soil conditions.

Measurements

Phenology of seedlings

Lucerne emergence was considered complete when both cotyledons were visible and had unfolded (Moot *et al.* 2000). The number of emerged seedlings was

Table 1 Soil test results for the trial site at the initial experimental setup on 8 October 2014.

Site	K	Ca	Mg	Na	P	S(SO ₄)	pH
			m.e./100 g soil		mg/L	mg/kg	in water
North	0.28	7.2	0.86	0.13	13	3	5.9
South	0.39	7.0	0.76	0.12	16	3	5.8
Lower optima	0.26	-	0.34	-	15 - 20	11	5.3

Note: Soil tests were evaluated using the Ministry of Agriculture and Fisheries Quick Test (MAF QT) procedures. Lower optima for plant growth according to (Morton & Roberts 1999).

counted from two 1 m drill rows in each plot. Counts were made every 1-3 days until seedling number became constant for three consecutive dates. Appearance of primary leaves was measured on five tagged stems per genotype per plot. Starting from seedling emergence, these tagged stems were assessed every 1-3 days for (i) the number of fully expanded primary leaves, (ii) stem height (from ground to apical bud) and (iii) the number of axillary leaves (branching) at each main-stem node.

Seedling shoot and root biomass

Seedling shoot and root biomass were assessed when 50% of the tagged seedlings had reached the three and six trifoliolate main-stem leaves. A single 20 cm long section of drill row in each plot was excavated intact. In the laboratory, shoot and root fractions in each section were separated. The root fraction included the entire root system. Shoot dry matter (DM) measurements were also taken every 14 days, starting when more than 50% of the tagged seedling plants had initiated buds (visible buds) until open flower. At the end of the seedling period (when 80% of the stems had an open flower), crown and taproot DM were sampled. Shoot samples were initially harvested from a single 0.2 m² quadrat per plot using hand shears to cut all shoots just above crown height (50 mm from ground to crown). A root sample (crowns + taproots) was then obtained from the same quadrat by digging a trench to a depth of 300 mm. Materials were washed clean of soil and all shoot and root samples were dried in a forced-air oven set to 60°C until a constant weight was achieved.

Canopy expansion and light interception

Crop leaf area were measured from a subsample of 20 stems. Leaves were removed from stems and the area of green lamina was quantified by a leaf area meter (LICOR 3100; Licor Inc. Lincoln, USA). The measurement process occurred on three occasions when the shoot DM was measured.

The amount of incident radiation above (R_0) and transmitted radiation below (R_i) the canopy was measured directly, non-destructively using a Sunscan plant canopy analyser (Delta-T Devices Ltd, Burwell, Cambridge, England). R_0 and R_i measurements were taken every 7 days, starting 45 days from sowing, when canopy height was above the sensor height (30 mm). Seven above and below canopy readings were taken per plot for incident and transmitted radiation.

Thermal time accumulation

Daily thermal time (Tt, °Cd) was calculated by using a broken-stick threshold model (Jones & Kiniry 1986) where Tt is assumed zero for air and soil temperatures ($T_{air, soil}$) below the base temperature (T_b) of 1.0 °C (Moot *et al.* 2001). For temperatures less than 15°C,

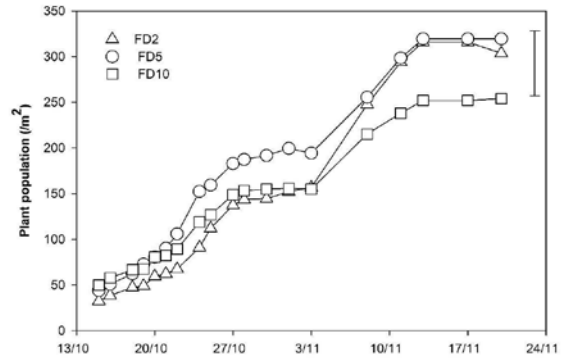


Figure 2 Field emergence over time of lucerne seedlings with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represents the LSD ($\alpha=0.05$) for the final emergence population.

Tt is accumulated linearly at a rate of 0.7 °Cd/°C and then at a rate of 1.0 °Cd/°C until the optimum temperature (T_{opt}) of 30 °C was reached (Moot *et al.* 2001; Teixeira *et al.* 2011). This method calculates Tt at hourly intervals which are integrated over one day. Thermal time accumulation was calculated as the sum of daily Tt using soil temperatures for emergence and air temperatures for leaf appearance.

Phyllochron

The phyllochron (°Cd/ primary leaves) was calculated as the slope of the linear relationship between the primary numbers of leaves on tagged stems and accumulated thermal time for each cultivar.

Statistical analyses were performed using GenStat 16th edition (VSN International). The mean values were compared using Fisher's least significant difference, LSD (at $\alpha = 0.05$).

Results

Lucerne establishment

Final emergence of FD2, FD5 and FD10 at 303, 319 and 255 plants/m², respectively (Figure 2). Based on their respective sowing rates of 577, 490 and 450 seeds/m² these represented 52%, 65% and 56% emergence. All genotypes showed two phases in emergence with an initial 150-200 plants/m² emerging up to 27 October 2014. During November, rainfall of 50 mm (Figure 1) induced a second phase of emergence which resulted in a final population of more than 250 plants/m². This is considered sufficient for high yielding crops in this environment (Moot *et al.* 2012). Neither the number of days from sowing to 50% emergence ($P=0.19$) nor the accumulated thermal time for 50% emergence ($P=0.20$), which averaged 263°Cd, differed amongst the three genotypes.

Table 2 shows the seedling shoot and root biomass of all three crops was not different at the third ($P=0.90$) or

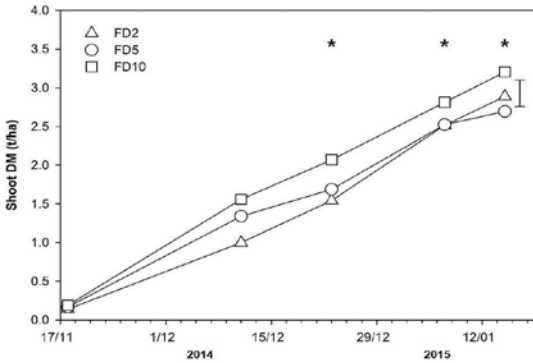


Figure 3 Shoot dry matter (DM) yield of lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final harvest. * = $P<0.05$.

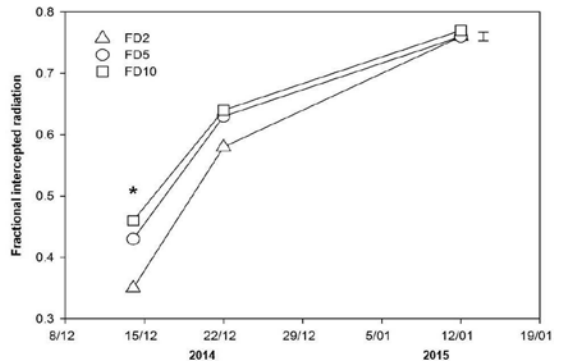


Figure 5 Fractional interception of radiation against time of three lucerne genotypes with different fall dormancy (FD) ratings sown on 08 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final measurement. * = $P<0.05$.

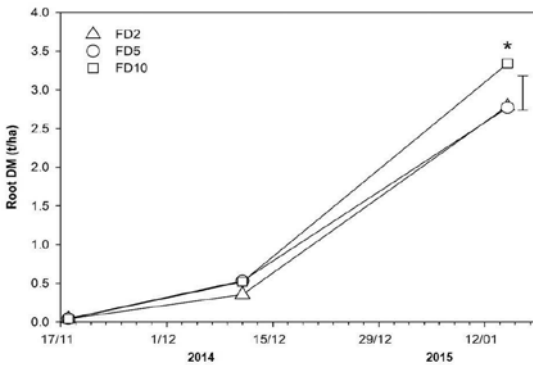


Figure 4 Root dry matter (DM) of three lucerne genotypes with different fall dormancy (FD) ratings sown on 08 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final harvest. * = $P<0.05$.

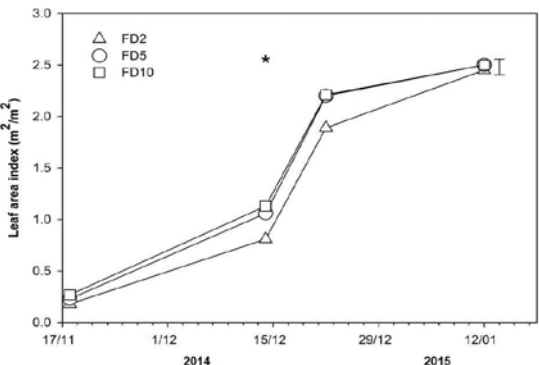


Figure 6 Leaf area index of three lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final measurement. * = $P<0.05$.

sixth ($P=0.22$) trifoliolate stages. The exception was for the initial fraction of biomass partitioned to root (P_{root}) which was lowest ($P<0.01$) for FD10 at 0.17 at the third trifoliolate stage. The shoot yields calculated from these

Table 2 Shoot and root plant dry weight (mg/plant) and proportion of partitioning of biomass to roots (P_{root}) for seedling lucerne genotypes with different fall dormancy (FD) ratings at the 3rd and 6th trifoliolate leaf stage from sowing on 8 October 2014.

Lucerne genotypes	18/11 3 rd trifoliolate leaf			11/12 6 th trifoliolate leaf		
	Shoot	Root	P_{root}	Shoot	Root	P_{root}
FD10	29.8	6.02	0.17 _a	482	154	0.25
FD5	28.0	7.22	0.21 _b	427	163	0.27
FD2	29.3	8.49	0.22 _b	386	142	0.26
P	0.90	0.08	<0.01	0.22	0.60	0.21
SE	3.94	1.07	0.01	53.9	20.81	0.02

seedling measurements and subsequent quadrat cuts show that the FD10 genotype had the highest ($P=0.03$) shoot yield at the end of the seedling phase at 3.2 t DM/ha. The separation of yield was visually apparent in the field by early December (6th trifoliolate stage) but only significant at the final harvest (Figure 3). The root biomass of the FD10 (3.3 t DM/ha) cultivar was also higher ($P=0.02$) than the others at the final harvest (Figure 4), but P_{root} (0.50) was not different. Root and shoot yields at the end of the seedling phase were 6.55 t DM/ha for FD10 compared with 5.57 t DM/ha for FD2 and FD5.

In the early seedling growth period the FD2 crop had the lowest fraction of intercepted radiation (0.35) but there were no differences at the end of the seedling phase (Figure 5). At the final harvest the fraction of intercepted radiation was still less than 0.78. This means the leaf area index was always below the critical level (LAI_{crit}) of 3.2 (Teixeira *et al.* 2011) required to intercept 95% of available radiation (Figure 6). The LAI showed a rapid

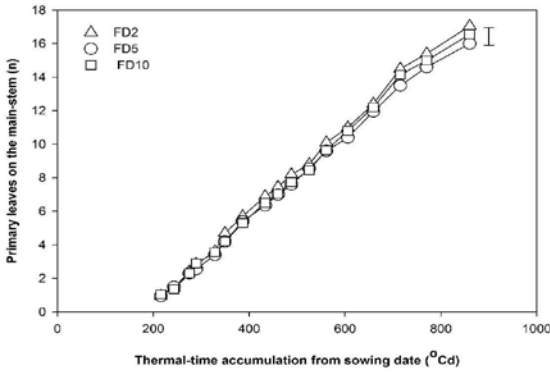


Figure 7 Number of primary leaves on the main-stem of three lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final total number of primary leaves.

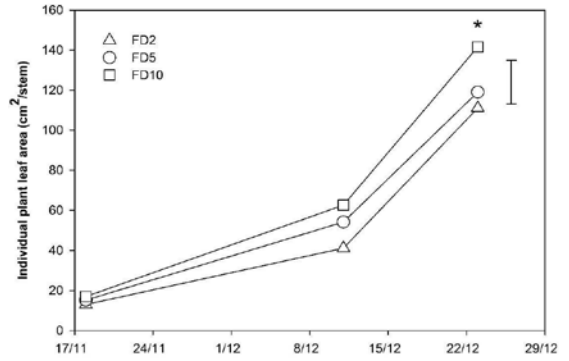


Figure 9 Leaf area per stem of three lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final measurement. * = $P<0.05$.

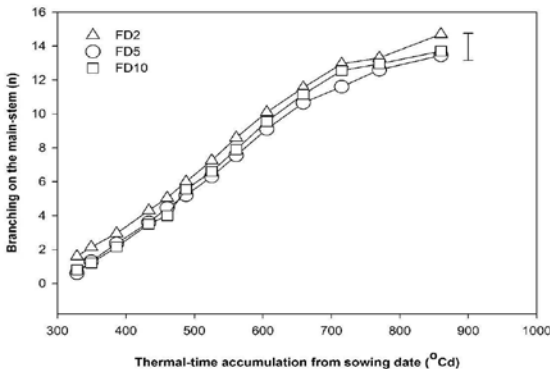


Figure 8 Number of branches on the main-stem of three lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final total number of branches.

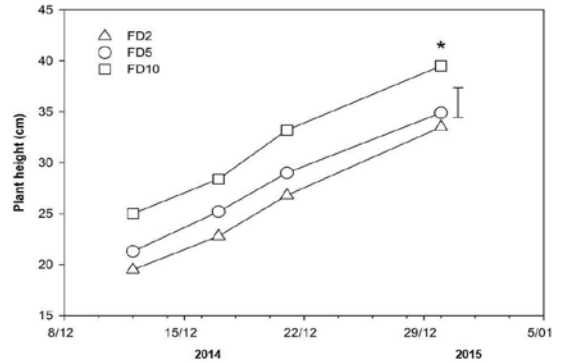


Figure 10 Plant height of three lucerne genotypes with different fall dormancy (FD) ratings sown on 8 October 2014. Note: Error bar represent LSD ($\alpha=0.05$) for the final measurement. * = $P<0.05$.

increase between sampling on 15 and 23 December 2014. This may reflect water stressed crops with wilted canopies until 3.1 mm of rain restored turgor pressure on 21 December 2014. The number of leaves on the main stem was also consistent among genotypes. Each genotype expanded up to 17 leaves by the final harvest (Figure 7). Branching was not different ($P=0.21$) among the genotypes (Figure 8). The phyllochron for all genotypes across the seedling phase was 52°Cd per primary leaf ($P = 0.06$). In contrast, individual leaf area differed ($P<0.05$) among genotypes being 142, 119 and $111 \text{ cm}^2/\text{stem}$ for FD10, FD5 and FD2, respectively (Figure 9). Furthermore, plant height at the open flower stage was greater ($P<0.05$) at 39.5 cm for FD10 compared with 34.5 cm for FD5 and 33.5 cm for FD2 (Figure 10).

Discussion

The collective results of seedling growth and development suggest some similarities but also important differences among these lucerne genotypes

with different FD ratings. Thermal time requirements for emergence were not different and consistent with previous reports (Sim *et al.* 2015). A second flush of emergence (Figure 2) with rain has previously been reported (Wigley *et al.* 2012) for lucerne and may indicate an effect of seed coating or an innate population response to different soil temperature and moisture conditions (Sharifiamina *et al.* 2016). Despite differences in the temporal pattern of emergence all crops established at sufficiently high plant populations (Teixeira *et al.* 2011) to maximise yields.

The early shoot and root growth, between the third and sixth trifoliate leaf stages, also showed no differences amongst the genotypes (Table 2). The implication was that ontogeny was consistent across genotypes and independent of any environmental signals or FD rating. However, the pattern of partitioning for FD10 showed a lower proportion of biomass in roots at the third trifoliate stage. This suggests the priority for this genotype was shoot over root production at this early

stage. By the end of the seedling phase (15 January) priority of shoot growth for FD10 had resulted in a higher fraction of intercepted radiation (Figure 5), particularly compared with FD2. As a consequence of more radiation intercepted, it had 20% higher shoot and 16% more root biomass than FD2.

The cause of the yield difference appears related to the development of individual leaf area per plant (Figure 9) and plant height (Figure 10). The taller FD10 genotype produced thicker leaves, with a higher specific leaf weight of 0.0068 (g DM/cm²) compared with 0.0065 for FD5 and 0.0063 for FD2 ($P=0.02$). This implies that the leaf arrangement on these taller plants allowed greater light interception, particularly as the seedling canopy was below the critical leaf area index for the whole growth cycle (Figure 6). Light interception is often the key limitation to crop and pasture establishment because water and nutrients are provided to ensure the maximum opportunity for success (Fick *et al.* 1988). For FD10 this strategy of increased height and leaf area resulted in the higher shoot (Figure 3) and root (Figure 4) biomass at the end of the seedling phase. Given the highest proportion of light interception was only 0.7, the crops were all below critical leaf area index for all of the seedling growth period. This means the advantage in leaf area for FD10 was cumulative throughout this establishment phase and the most successful strategy to maximise seedling growth (Figures 9 and 10). The results also suggest the advantage in leaf area was not associated with leaf appearance. The phyllochron was not different among genotypes and consistent with previously reported values (Teixeira *et al.* 2011).

A difficulty in non-destructive measurement of light interception with seedling crops is that the instruments require some canopy height and leaf area before accurate measurements can be taken. This prevented light interception measurement until 67 days after sowing (mid-December) (Figure 5). Despite this, the individual plant leaf area data coupled with these non-destructive measurements consistently highlighted the advantage in light interception to the FD10 crop. Similarly the separation in height measurements from mid-December (Figure 10) indicates the expression of taller plants from higher FD ranking occurred throughout the seedling phase. This provides an independent validation of the breeders FD rankings for these genotypes.

Species differences in their allocation of biomass to shoot and root are often used to define their success at establishment (Thomas 2003). Specifically, the lack of competitive ability of Caucasian clover has been linked to its disproportionate allocation to roots at the expense of shoots (Black *et al.* 2002). Differences among genotypes are less well documented. The FD rating for lucerne is based on height with an emphasis on fall

or autumn growth. However, these results suggest the height and leaf area advantage for FD10 were expressed after spring sowing and this resulted in greater seedling growth. It remains to be seen if this yield advantage is apparent during future regrowth crop cycles.

Rainfall during October was half that normally expected (Figure 1). However, the cultivated seedbed had provided sufficient soil moisture for initial emergence to commence. The decision not to irrigate during this phase was made because a reasonable population had emerged. These early emerged plants formed the basis of the samples taken for the third trifoliolate measurement. The subsequent second flush of emergence after November rainfall meant there were plants of different sizes and ages in the seedling samples taken at the sixth trifoliolate stage. The tagged plants all came from the initial phase of emergence so time of sampling was presented accurately. The different aged plants were not recorded separately and may have affected the result at the sixth trifoliolate stage and resulting in no significant difference in shoot yield until the end of the cycle. Agronomically, it seems likely that these later emerged plants were smaller and potentially will be the first ones to die in the intense self-thinning process that occurs in establishing lucerne stands (Teixeira *et al.* 2007a; Moot *et al.* 2012). It follows that management decisions should be made on the basis of the growth and development of the oldest plants in the crop, as occurred in this study.

Conclusion

Shoot and root DM production of the least dormant lucerne genotype (FD10) were higher than the other two genotypes (FD2 and FD5). Higher DM production of FD10 was associated with higher light interception at the early stages of crop establishment caused by higher LAI expansion rates. The FD10 genotype also showed a distinct morphology with taller stems and increased light interception. Thermal time to emergence, fractional partitioning of biomass to roots, leaf appearance and branching rates were similar among the three genotypes. These results indicate that the growth components of genotype (leaf area expansion and stem elongation) were most closely correlated with autumn or fall dormancy ratings during spring seedling growth. In contrast, the development (emergence and phyllochron) was consistent among genotypes.

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