Establishment of lucerne *(Medicago sativa)* sown on five dates with four inoculation treatments

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Abstract

The establishment and growth of 'Stamina 5' lucerne *(Medicago sativa)* seed sown with three inoculant carriers (ALOSCA®, coated, and peat slurry treated) or as bare seed (control) on five dates (21 October 2010, 9 November 2010, 8 December 2010, 13 January 2011 and 3 February 2011) was investigated on a Lismore stony silt loam soil at Ashley Dene dryland research farm in Canterbury. Initial lucerne populations were 300 plants m^{-2} from coated seed and on average 200 ± 11.2 plants m^{-2} from bare seed, ALOSCA® and the peat slurry inoculated seed. The higher population from coated seed treatments did not confer a herbage yield advantage. In the establishment year, yield was lowest (0.59 t DM ha^{-1}) from the last sowing (3 February 2012) and highest from sowing dates (SD) 2 and 3 (2.6 ± 0.12 t DM ha^{-1}). Yields were restricted by the low volumetric soil moisture content from November until March. The declining autumn photoperiod (14.9 to 14.1 hours) probably increased the partitioning priority of assimilates to the roots, reducing the above ground DM in the later sowing dates. In most cases, DM yields in Year1 were unaffected by seed inoculant treatments. In Year 2, DM production from the peat slurry treated seed (8.0 t ha^{-1}) was highest, while coated seed crops were lowest (6.0 t ha^{-1}). The effects of sowing date carried through to the second year with lower DM yields from SD4 and SD5 (6.0 ± 0.18 t ha-1) compared with 7.3 ± 0.18 t ha-1 from the earlier sowing dates.

Keywords: alfalfa, ALOSCA®, bare seed, coated seed, *Ensifer meliloti*, peat slurry treated seed, rhizobia, sowing date

Introduction

Lucerne is a pasture species suited to dryland conditions. Its deep taproot allows it to extract water from deeper soil layers and use it more efficiently than grass-based pastures (Moot *et al.* 2008). Lucerne also fixes its own nitrogen once it has formed a symbiotic relationship with rhizobia bacteria (Wynn-Williams 1982). Lucerne is likely to become increasingly important to dryland farmers as soil moisture deficits along the east coast of the South Island are predicted to increase (Salinger 2003).

Successful inoculation with effective rhizobia is an essential factor in the successful establishment of legumes. Inoculants have been found to increase lucerne yields by 15–900% (Burton 1972) and historically there has been little debate on the need for inoculants on the majority of agricultural soils (Allen & Allen 1958; Burton 1972). There are several commercial products available for lucerne inoculation but the comparative advantages of each have not been established. Furthermore, a recent review has questioned the need for inoculation of other pasture legume species such as white clover in New Zealand (Lowther & Kerr 2011). The objective of this study was to examine the efficacy of four different forms of delivery of Rhizobia *Ensifer meliloti* inoculants and the effects on lucerne establishment and growth.

Materials and Methods

Experimental site

The experiment was established at the Lincoln University dryland research farm, Ashley Dene (43° 45° 39' S and 172° 19' E, 38 m a.s.l.) on a Lismore stony silt loam soil with a depth of 0.45–0.75 m above alluvial gravels (Webb & Bennett 1986), with moderate fertility (Table 1). The paddock had been sown in lucerne from 1982–1987, 1988–1998 and 1999–2006. It was then sown in turnips and 'Grasslands Moata' annual ryegrass for 2 years. The forage crop was grazed off over the winter of 2010. On 14 September 2010 lime was applied at 4 t ha^{-1} with 200 kg ha^{-1} of superphosphate. The site was then ploughed, harrowed and rolled in late September 2010. A pre-plant herbicide, Treflan®, was applied (0.8 kg trifluralin ha^{-1}) and soil incorporated on 1 October 2010 to control weeds.

Experimental Design

The experiment used a split-plot design, with five sowing dates (SD1: 21 October 2010, SD2: 9 November 2010, SD3: 8 December 2010, SD4: 13 January 2011 and SD5: 3 February 2011) as main plot factors and three inoculation carriers (peat, coated, and ALOSCA®) plus a bare seed control as subplots. There were four replicates.

Inoculation and sowing

The Australian bred lucerne cultivar 'Stamina 5' with

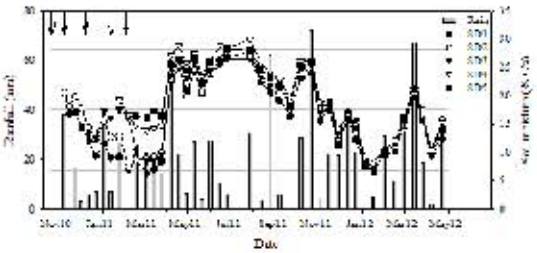

Figure 1. Rainfall (bars) and soil moisture readings (% v/v, 0–0.2 m) at Ashley Dene, Canterbury. Arrows indicate the five lucerne sowing dates (SD1 = 21 Oct 2010, SD2 = 9 Nov 2010, SD3 = 8 Dec 2010, SD4 = 13 Jan 2011 and SD5 = 3 Feb 2011) for. The solid grey lines are: field capacity (28.1%, top), critical deficit (17.5%, middle) and the estimated, field derived, lower limit (7%, bottom) to plant water extraction (R. Sim unpublished data).

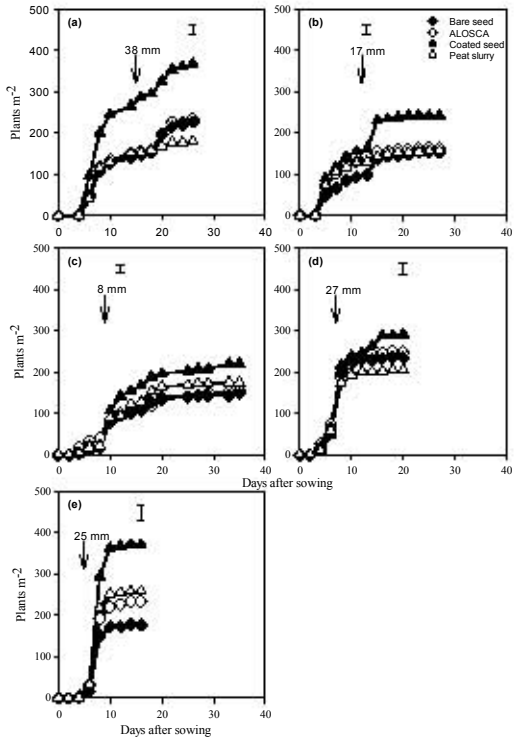

Figure 2. Number of seedlings emerged after sowing on five dates (a) 21 Oct 2010, (b) 9 Nov 2010, (c) 8 Dec 2010, (d) 13 Jan 2011 and (e) 3 Feb 2011. Seed treatments were a bare seed control, lime coated seed, ALOSCA® granules or peat slurry inoculant at Ashley Dene, Canterbury. Error bars represent the largest standard error of the mean for all measurement dates. Arrows indicate first significant rainfall (>7 mm) after sowing.

a dormancy rating of 5 was sown. This cultivar has recently been introduced into New Zealand for use on farms. Lucerne was sown as i) bare seed at 10.5 kg ha^{-1},

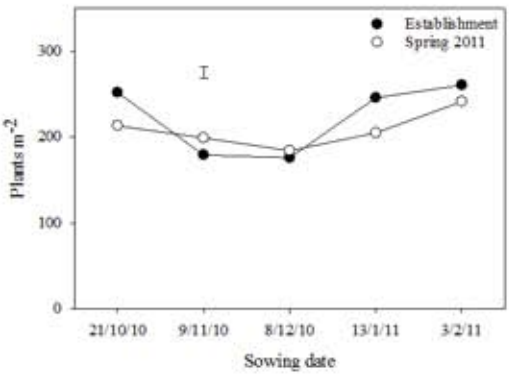

Figure 3. Change in plant population (plants m^{-2}) over two measurement dates (establishment and then spring of the second year) of 'Stamina 5' lucerne established at Ashley Dene, Canterbury on five different dates (21 Oct 2010, 9 Nov 2010, 8 Dec 2010, 13 Jan 2011, 3 Feb 2011). Error bar is SEM for the SD × time interaction.

ii) coated with a peat slurry and *E. meliloti* iii) or seed was sown with bentonite clay granules (ALOSCA®) that contained *E. meliloti* (Carr *et al.* 2006). ALOSCA® was mixed with bare seed at the recommended rate of 10.5 kg ha^{-1} in the drill at sowing. iv) Coated seed containing *E. meliloti,* a contact fungicide against *Pythium* spp., molybdenum and lime was drilled at 16.0 kg ha^{-1} which equated to the bare seed sowing rate. An Øyjord cone seeder, with 0.15 m row spacing, was used to sow 4.2 × 7 m plots (29.4 m^2) with 0.5 m gaps between plots. Excess seed of each treatment was removed from the drill hoppers using compressed air to prevent cross contamination. The sowing order was: bare seed, ALOSCA® mix, coated seed and then the peat slurry mix.

Rainfall and soil moisture

Total monthly rainfall and volumetric soil moisture content (0–0.2 m soil depth) were measured on site. Rainfall was measured using a rain gauge and volumetric soil moisture content with Time Domain Reflectometry (Trace Systems, Model 6050X1) in the coated seed treatments. Soil moisture measurements were made at 7–33 day intervals from the first sowing date until the end of the second growth season (20 April 2012). Figure 1 shows the soil moisture content (% v/v) and rainfall between sequential measurements. The critical deficit, or point beyond which growth declines because of water stress, occurs when <50% of plant available water remains (Penman 1971). In the top 0.2 m of the soil, the field derived field capacity was 28.1% and lower limit of plant available water was 7% (R. Sim, unpublished data). Thus, the critical deficit was about 17.5%.

In the establishment year, soil moisture was below

17.5% from 16 November 2010 to 25 March 2011 for SD1, from 13 December 2010 to 25 March 2011 for SD2 and SD3, from 6 February 2011 to 25 March 2011 for SD4, and from 17 February 2011 to 25 March 2011 for SD5. In Year 2, water stress generally began in early November. Soil under all crops remained at ≤17.5% v/v from 28 November 2011 to mid-February 2012.

Measurements

Seedling emergence, initial and established plant populations

Emergence was defined as the time of spade leaf appearance on the seedling. Two 1 m drill row lengths per plot were randomly located and marked for observation every second day from sowing until no further plants emerged. Seedling counts in the marked rows provided estimates of the "initial population". The "established populations" were measured in the spring of the second season by counts of taproot number in a 0.2-m deep trench dug alongside 1 m of drill row. The soil around the taproots was then loosened to enable accurate counting.

Dry matter yield

After sowing, each seedling crop was left to flower to optimise plant establishment (Teixeira *et al.* 2011). After these seedling crops, there were two regrowth cycles for SD1 and SD2, one regrowth cycle for SD3 and SD4 and none for SD5. At the end of each cycle destructive harvests were made from a 0.2 m^2 quadrat cut to ground level. Remaining herbage was then mown to stubble height of ca. 5 cm and removed from the site. Additional mid-cycle harvests or plate meter readings were also made but are not included in the analysis of yield. Dry matter (DM) yields were determined by drying in a forced air oven for a minimum of 48 hours at 65°C.

In Year 2 (1 July 2011 – 20 April 2012, 294 days), there were four regrowth cycles for all treatments. These ended on 27 October 2011, 15 December 2011, 1 March 2012 and 20 April 2012. Destructive harvests were taken at the end of three regrowth cycles but no measurement was made before mowing in the third cycle (1 March 2012) which was severely water stressed. For this period an early mid-cycle measurement (30 January 2012) was used. Additional data collected from the coated seed pastures (not presented) indicated that DM yields between 30 January 2012 and 1 March 2012 remained constant.

Statistical Analysis

Treatment effects were analysed by split plot ANOVA in GenStat (13th Ed, VSN International Ltd.). Fisher's protected least significant difference (LSD) tests were used to separate means when $P \leq 0.05$. Repeated measure analysis was used to quantify changes between "initial" and "established" plant populations. This analysis partitions effects over time when variables are not necessarily independent of each other.

Results and Discussion

Unless otherwise stated there were no interactions between sowing date and inoculation treatment.

Initial and established populations

Initial lucerne seedling populations were 43% higher ($P<0.005$) on average for SD1, SD4 and SD5 (253 ± 16.1) than for SD2 and SD3 (177 ± 16.1) in Year 1. Seed treatment also affected initial populations. Coated seed plots had 300 emerged plants m^{-2} (60% of the 490 seeds m^{-2} sown) compared ($P<0.001$) with peat, ALOSCA® and the bare seed control which had on average only 200 ± 11.2 plants m^{-2} or 40% emergence of sown seed (Figure 2). A study by Horikawa & Ohtsuka (1996) also found that lime coated lucerne seed experiences a rapid degree of nodulation and early seedling growth compared with peat slurry inoculated seed. For SD1, SD2 and SD4, coated seed showed a two-step emergence pattern which corresponded to rainfall events. Specifically, for SD1 38 mm of rain fell 15–16 days after sowing (DAS), SD2 received 17 mm of rain 12 DAS and for SD4 there was 27 mm of rain at 5–9 DAS. The second flush of emergence was the main cause of the higher initial populations from coated seed crops except for SD1 where coated seed crops showed higher emergence from sowing.

When re-measured in spring 2011, established plant populations differed between the seed treatments. In coated seed plots there were 267 plants m^{-2} or 41% more ($P<0.001$) than the average of the other three seed treatments (189 ± 10.2 plants m^{-2}). All of these establishment populations were above the minimum of 43 plants m^{-2} required to maximise yield (Teixeira et al. 2007a). The decline between emergence and the spring 2011 establishment counts is consistent with previous reports that crops with higher initial populations have the highest plant mortality after emergence and the lowest final plant populations as a percentage of seed sown (Sims 1975). However, self-thinning is often compensated for by an increase in the number of stems per plant. For example, Teixeira *et al.* (2007a) reported that lucerne plant populations at Lincoln declined from 130 m^{-2} in June 2002 to 60 m^{-2} in September 2004 due to natural self-thinning, but yield was unaffected because of increased stems per plant.

Repeated measure analysis showed there was a SD × time interaction ($P<0.05$) for plant population (Figure 3). The interaction was caused by a 16% reduction in

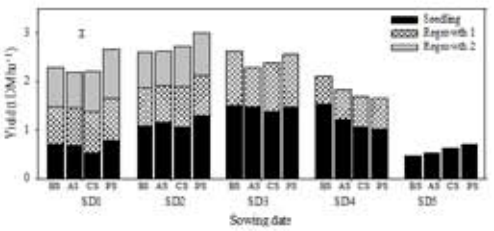

Figure 4. Total annual yield (t DM ha^{-1}) in the first year of 'Stamina 5' established on five dates (21 Oct 2010, 9 Nov 2010, 8 Dec 2010, 13 Jan 2011, 3 Feb 2011) and with four seed treatments: bare seed (BS), ALOSCA granules (AS), coated seed (CS) and peat slurry treated (PS) at Ashley Dene, Canterbury. Details of harvest dates and durations were presented in Table 2. Error bar is the SEM for the main effect of sowing date on total DM yield.

plant numbers between the initial population counts and the counts in spring 2011 in SD1 and SD4. In contrast for SD2, SD3 and SD5, there was no difference between initial and established plant populations. SD5 had a high initial plant population without the same decrease in plant numbers as recorded for SD1 and SD4. This is possibly because SD5 emerged later in the growing season, resulting in smaller plants with fewer leaves causing less self-thinning in the crop between emergence and establishment.

Dry matter yield – Year 1

As expected, DM yield in Year 1 differed between sowing dates with SD2 the highest (2.7 t DM ha^{-1}) and SD5 the lowest (0.6 t ha^{-1}) ($P<0.001$) (Figure 4). Because the first harvest was made after different times for each sowing date, it was not possible to compare production at a given date for seedling crops. Their yields differed ($P<0.001$) due to the differences in the main environmental drivers of temperature, photoperiod and soil moisture that each seedling crop was exposed to. SD3 produced the highest ($P<0.001$) yield as a seedling crop of 1.6 t DM ha^{-1} over 107 days. This was more than double the 0.6 ± 0.07 t DM ha^{-1} produced from seedling crops in SD1 and SD5 which had grown for 95 and 131 days, respectively.

The highest total accumulated DM yields over Year 1 were produced in SD2 and SD3 plots. This was due to these crops having more time to accumulate DM in the first season. However, SD1, SD2 and SD3 all had lower yields than expected because soil moisture content dropped below 17.5% (0–0.2 m soil depth) in late November and remained there until early April (Figure 1). The later planting of SD4 and SD5 ensured soil moisture and temperatures (19.7°C for SD5) were adequate for seedling crops, but the cooler autumn temperatures meant thermal time accumulated more slowly. Lucerne also partitions a higher proportion of

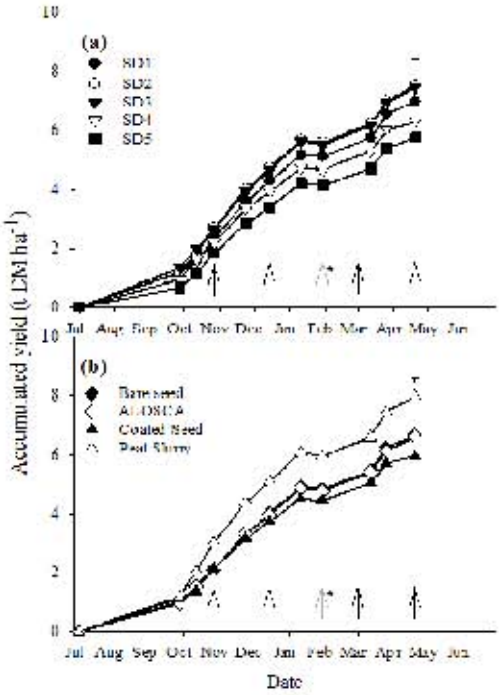

Figure 5. Accumulated DM production of 'Stamina 5' lucerne in Year 2 showing main effects when sown (a) at five different dates (21 Oct 2010, 9 Nov 2010, 8 Dec 2010, 13 Jan 2011, 3 Feb 2011) and (b) with four seed treatments: bare seed (BS), ALOSCA granules (AS), coated seed (CS) and peat slurry treated (PS). Black arrows indicate end of the four regrowth cycles. Grey arrow (*) indicates use of non-destructive yield data as harvest not made at the end of the regrowth cycle. Error bars are SEM for the main effect shown.

carbon and nitrogen to its roots in the autumn (Teixeira *et al.* 2007b). Thus, the declining photoperiods (14.9 to 14.1 hours) probably also increased the partitioning priority to the roots, reducing above ground DM production.

The bare seed control followed the same growth and production trends as the inoculated crops. Gandee *et al.* (1999) also reported that DM production did not differ between inoculated and un-inoculated plants. In the present study these findings could be due to differences in the level of nitrogen mineralisation (Table 1) between SD1 and SD5 (42 versus 100 kg N ha^{-1}). This could have maintained crop growth in Year 1 with little requirement for fixed nitrogen. Assuming the lucerne crops were ca. 3% N (Brown & Moot 2004), the 100 kg N ha^{-1} mineralised was sufficient to grow the highest yielding crop (2.7 t DM ha^{-1}) in Year 1. Therefore, it is possible that crops did not require N fixation to meet N demand. High levels of available soil nitrogen have also been found to have an inhibitory effect on N fixation (Munns 1967). This may be why the inoculated seed

did not confer an advantage over the bare seed control. Alternatively, indigenous rhizobia present in the soil could have successfully nodulated the plants grown from bare seed. Plating and genotypic characterisation of rhizobia from lucerne plants grown from bare seed found that these plants had successfully been nodulated with indigenous rhizobia species already present in the soil (Wigley 2011). These indigenous rhizobia could have been present in the soil as lucerne was grown on the site up until 2006.

Peat slurry treated seed had the highest yields for SD1 and SD2 (Figure 4). This could be linked to higher nodulation, N fixation and growth. However, culturing, DNA extraction and genotypic characterisation of rhizobia from lucerne plants grown from peat slurry, ALOSCA® and coated seed treatments found all had been successfully nodulated with *Ensifer meliloti* (Wigley 2011).

Dry matter yield – Year 2

In Year 2, DM yield differed between sowing dates with SD1–SD3 the highest (7.3 ± 0.18 t DM ha^{-1}) and SD4–SD5 the lowest (6.0 ± 0.18 t ha^{-1}) (P<0.001) (Figure 5a). These yield differences showed the effects of late sowing continued into the second year. For SD4 and SD5 this was probably because the delay restricted their ability to accumulate underground reserves in Year 1. Given "initial" plant populations (Figure 3) for SD4 and SD5 were >245 plants m^{-2}, this suggests the later sown lucerne remobilised less carbon and nitrogen to spring regrowth in Year 2. Teixeira *et al.* (2007b) have shown that spring regrowth is highly dependent on perennial nitrogen reserves. SD4 and SD5 lucerne continued to develop their root systems in the second year, probably reducing the allocation of carbon for shoot production (Teixeira et al. 2011).

DM yields differed (P<0.001) between the sowing dates for all four regrowth cycles. In the first spring regrowth cycle yield was 2.7 ± 0.10 t DM ha^{-1} from SD2 and SD3 and was lowest from SD5 (1.9 t ha^{-1}). For regrowth cycles 2, 3 and 4 the DM yields from SD1, SD2 and SD3 were greater (P<0.001) than the yield produced from SD4 and SD5.

Seed inoculation also affected DM yields in Year 2 (Figure 5b). Specifically, peat slurry treated seed produced 8.0 t DM ha^{-1} compared (P<0.001) with 6.7±0.16 t ha^{-1} from ALOSCA® and bare seed. Despite the coated seed having the highest number of established plants, its yield of 6.0 t DM ha^{-1} was the lowest in Year 2. Teixeira *et al.* (2007a) found that increased sowing rate and emergence populations will not necessarily increase DM production. They estimated that a minimum plant population of 43 plants m^{-2} was required to maintain a productive crop. In Year 2 the pastures resulting from seed treated with peat slurry produced the highest (P<0.001) yields in three of the four regrowth periods. At the harvest on 30 January 2012, yields from peat and bare seed treatments were superior (P<0.001) to those of ALOSCA® and coated seed. This result requires further investigation, but may indicate a higher rate of fixed N leading to higher photosynthesis rates in the crops from seed treated with peat slurry.

Based on the results from both years, it seems the

Table 1. Soil test results (0–0.15 m) for paddock M2B at Ashley Dene, Canterbury prior to each sowing date of 'Stamina 5' lucerne.

Sowing Date	pH (H_2O)	Olsen P (mg/l)	Potassium (me/100 g)	Sulphur (me/100 g)	Mineral-N (mg/kg)	Total N (kg/ha)
21 October 2010	6.0	14	0.24	10	38	42
9 November 2010	6.0	14	0.26	10	39	44
8 December 2010	6.0	15	0.22	11	64	71
13 January 2011	5.9	14	0.22	10	91	102
3 February 11	6.1	17	0.25	13	90	100

Table 2. Date of individual harvests for each of the five sowing dates in the establishment year and duration (days) of the establishment year. The value in brackets after the harvest date is the individual regrowth duration (days).

Sowing date	Rotation 1 (Seedling)	Rotation 2 (Regrowth 1)	Rotation 3 (Regrowth 2)	Duration of establishment season (days)
21 Oct 10	24 Jan 11 (95)	7 Apr 11 (73)	14 Jun 11 (68)	236
9 Nov 10	11 Feb 11 (94)	27 Apr 11 (75)	14 Jun 11 (48)	217
8 Dece 10	25 Mar 11 (107)	14 Jun 11 (81)	-	188
13 Jan 11	5 May 11 (112)	14 Jun 11 (40)	-	152
3 Feb 11	14 Jun 11 (131)	-	-	131

use of inoculants containing *E. meliloti* is not always necessary when establishing lucerne. However, it remains to be seen how long rhizobia can survive in the soil and whether adequate nodulation would occur if lucerne was introduced into soil that had not previously grown lucerne. Further research is also required to confirm the N fixing ability of the commercial inoculants compared with the indigenous rhizobia that formed many of the nodules in the current experiment.

Conclusions

- Coated seed had the highest initial lucerne populations (299 plants m^{-2}).
- Established lucerne plant populations were adequate in all seed treatments including the bare seed control.
- Sowing after the longest day (SD4 and SD5) reduced the shoot yield (6.0 t DM ha^{-1}) in Year 2.
- The yield advantage to the peat slurry treated crops in Year 2 requires further investigation.

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