Seed yield and subsequent emergence pattern of subterranean clover cultivars in response to summer rain

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Abstract
At Lincoln University, Canterbury, seven subterranean cultivars rated in Australia as having different levels of 'hardseedeness' were established. Monocultures were sown in autumn and allowed to grow and set seed. Seed yields ranged from 340 to 1050 kg/ha. Heavy rain in early January 2016 resulted in a "false strike" of ≤ 4.0% of seeds during the subsequent dry February. A second emergence event in March also resulted in a "false strike" with a further 7 to 15% of total seeds lost. However, cultivars established >1000 seedlings/m^2 after early winter rain, which is considered adequate for future persistence. Emergence was consistent with Australian hardseededness rankings. Cultivars with hardseed ranks <4 may be more suitable for dryland systems in New Zealand due to their early emergence and the ability to exploit the late summer and autumn rains.

Keywords: *Trifolium subterraneum*, dryland, re-seeding, 'Antas', 'Coolamon', 'Denmark', 'Mount Barker', 'Narrikup', 'Rosabrook', 'Woogenellup'

Introduction
Subterranean clover (*Trifolium subterraneum*) is an winter annual that has been promoted as a dryland pasture legume to provide late winter-early spring feed before perennial white clover and lucerne increase their growth in spring (Brown *et al.* 2006). Subterranean clover is the most widely sown annual clover in New Zealand pastoral systems (Monk *et al.* 2016). However, the commercial cultivars available are imported from Australia and local information is required to estimate initial seed yield which contributes to its persistence in New Zealand dryland areas. Subterranean clover is named for its unique ability to bury its seed, which is an advantage for close grazing systems (Norman *et al.* 2006). An important seed yield component is seed size which differs among cultivars. For pure swards of subterranean clover a mean of 260 kg of seeds/ha has been suggested as the minimum initial seed yield required to ensure an effective (1000 plants/m^2) re-establishment (Smetham 2003). For an inland New South Wales Australian climate, Dear *et al.* (1993) proposed 700 kg/ha seed set was required to guarantee a legume dominant pasture. This is because autumn establishment is prone to "false strike" whereby seedlings that emerge from early rainfall events fail to establish due to subsequent dry spells (Dodd *et al.* 1995).

Persistence of subterranean clover is also affected by the degree of hardseededness (due to an impermeable seed coat), which differs among cultivars and across environments (Hudson *et al.* 2015). The Australian hardseededness rating system (0-10 scale) (Nichols *et al.* 2013) does not always reflect field emergence because it is affected by management, agronomic (Taylor 2005) and physiological traits (Nicotra *et al.* 2010). Dear *et al.* (1993) found that for 'Woogenellup' and 'Mount Barker' approximately 60 ± 10% of seeds produced emerged in the subsequent autumn. The remaining 40% provided the seed resource for future years. The seed bank can be added to if management allows further seed set in later years. This paper presents the initial seed yield and a quantitative analysis of the impact of "false strikes" on the seed bank and seedling losses of seven mid-late season flowering cultivars (Nichols *et al.* 2013) including 'Mount Barker', which is an older cultivar resident in many New Zealand dryland pastures.

Methods
Seven cultivars of subterranean clover were sown at the Field Research Centre, Lincoln University (43°38'57"S, 172°28'04"E, 11 m a.s.l.) on 16th April 2015, in a randomised block design with four replicates. The commercial seeds were purchased from local suppliers and their characteristics are summarised by Nichols *et al.* (2013). Initial individual seed weights, estimated from four replicates of 200 seeds were: 'Antas' (spp. *brachycalycinum,* 11.0 mg), and the ssp. *subterraneum* 'Coolamon' (6.0 mg), 'Denmark' (7.0 mg), 'Mount Barker' (9.0 mg), 'Narrikup' (9.0 mg), 'Rosabrook' (8.0 mg) and 'Woogenellup' (10.0 mg). The soil at the research site was a Wakanui silt loam (*Aquic Haplustept*, USDA Soil Taxonomy), classified as a Mottled Immature Pallic soil in the New Zealand Soil Classification with available water holding capacity of ~150 mm/m (Hewitt 2010). Olsen phosphorus level was 13 mg/L and pH (H_2O) was 5.4. The area was cultivated with a rotary hoe to create a fine seedbed before bare, non-inoculated seeds were broadcast by hand and raked into 4 m^2 plots. Sowing rates were 20 g of seeds/m^2 (or 20 kg/ha). Sufficient rain (~ 44 mm) for germination occurred on 28th April (Figure 1). The

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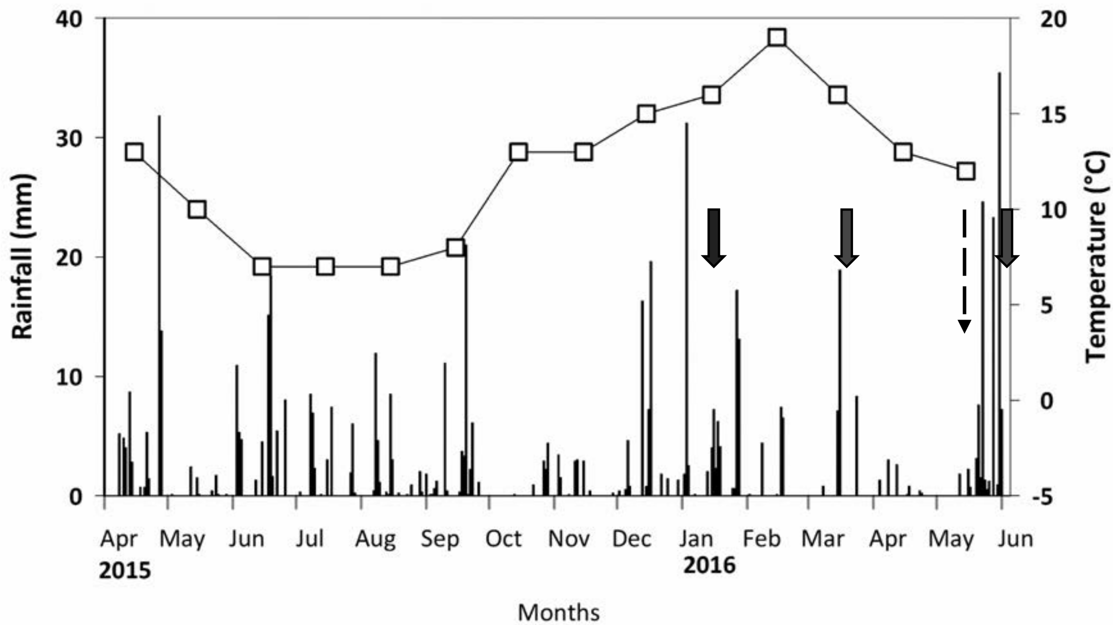

Figure 1 Daily rainfall (mm, vertical bars) and mean monthly air temperature (ºC) from April 2015 to July 2016 at Broadfields weather station, Lincoln University. Solid arrows indicate emergence assessment dates (13th January, 17th March and 1st June 2016). The dashed arrow indicates when 20 mm of irrigation water was applied (11th May 2016).

plots were mechanically cut on 28th September and 17th November 2015 at 20 mm height using a ride-on Walker® mower. The herbage was removed to simulate grazing. Plots were then left to set seed and dry out from late November.

Long-term annual average rainfall for the study area is 630 mm and the total accumulated rainfall during the experiment (April 2015 to June 2016) was 629 mm (Figure 1). Annual Penman potential evapotranspiration is 1095 mm and usually exceeds rainfall from September to April, with a long-term potential soil moisture deficit of 500 mm (NIWA 2017). Potential evapotranspiration exceeded rainfall from October to December 2015 (310 mm) and in early-February and April 2016 (213 mm). During the experiment the mean air temperature was 12ºC.

To estimate seed yield (kg/ha) subterranean clover burrs were sampled on 12th January 2016 with a metal corer (2 cores/plot, each 120 mm diameter) to 25 mm depth. Burrs and seeds were harvested from above- and below-ground and then processed manually. The number of burrs, seeds and the seed weight (mg) were recorded to estimate the percentage of burr burial and seed number/m^2.

Summer rain (~ 120 mm) fell from mid-December 2015 to early-January 2016 (Figure 1) and caused the first seed emergence (first "false strike") in some cultivars. The number of seedlings at cotyledon to the two trifoliate leaf stage was counted on 13th January in eight 10 x 10 cm quadrats in each plot and added to the seed number (seeds/m^2) to get the total seed density and seed yield values. Each plot was visually assessed for seedling mergence at four random points using a scale from 0-5. Emergence ratings were calibrated against seedling counts. Based on the January counts, the visual scale was adjusted to the range from 0 (no seedlings) to 5 (> 1500 seedlings/m^2). The mean values (Table 1) were then used to create a simplified linear function (seedlings/m^2 = 316x - 64, R^2 = 0.97, where x corresponds to the visual score value) to estimate subsequent seedling populations (Teixeira *et al.* 2017).

On 22nd January 2016, all plots were mown (50 mm height) to remove tall broadleaf (mainly *Rumex obtusifolius*, *Stellaria media*, *Taraxacum officinale*, *Chenopodium album, Polygonum aviculare* and *Achillea millefolium*) and grasses (*Lolium multiflorum* and *Phalaris aquatica*) weeds. Broadleaf weeds were controlled with imazethapyr applied at a rate of 72 g a.i./ha (300 ml/ha Spinnaker®) on 21st February. The emerged seedlings died (first "false strike") due to moisture deficit in late-February (Figure 1). New seedlings that had emerged were scored on 17th March 2016 (second "false strike") after accumulated rainfall of 90 mm (from 14th January to 17th of March). All weeds and subterranean clover were removed with glufosinate-ammonium herbicide (1000 g a.i./ha, 5L/ha Buster®) to assess a third emergence. The herbicide was applied on the 23rd March 2016. This created bare ground. A dry period in April (Figure 1) meant the area was irrigated on 11th May (~20 mm) to encourage a third period of seedling emergence during the last 2 weeks of May. The third emergence was quantified on the 1st June 2016.

The cumulative number of seedlings (seedlings/m^2) was subtracted from the total number of seeds produced/m^2 to calculate the number of seeds remaining ungerminated in the soil seedbank after the three emergence events. The percentage of seedlings lost was reported as seed losses over time (Equation 1).

Percentage of seed loss (%) = 100 x $\frac{a}{b}$

Where, 'a' is the cumulative number of emerged seedlings/m^2 and 'b' is the total number of seeds/m^2. It was assumed that the percentage of seed loss was only due to emergence as seed losses due to insect or diseases were not quantified in this study (Simpson *et al.* 2001). The measured data were plotted against the burr burial score values (0-10) of Nichols *et al*. (2013) and a positive correlation r = 0.90) was found. Results were analysed using one-way analysis of variance (ANOVA) using R software with Tukey's Honest Significance Test (THSD, α = 0.05). Minimum significant differences (MSD) are reported.

Results

There were differences (P<0.05) among cultivars in the percentage of buried burrs (Table 2). 'Antas' (brachycalycinum) had the lowest percentage of burr burial (40%) in contrast to 'Coolamon' and 'Narrikup' (subterraneum, > 70%).

The number of burrs/m^2 was almost 40% higher (P = 0.04) for 'Mount Barker' than the other cultivars. The number of seeds in each burr was also affected (P<0.001) by cultivar.

There was a positive correlation (r = 0.80) between individual seed weights of harvested seed and initial sown seed weights (Figure 2). The individual seed weight (mg) measured was also correlated (r = 0.90)

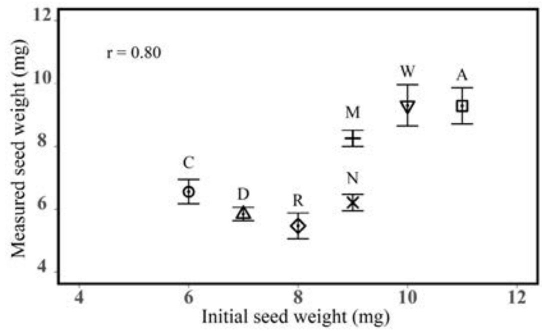

Figure 2 Mean measured seed weight (mg) against the initial seed weight (mg) for subterranean clover cultivars 'Antas' (□), 'Coolamon' (○), 'Denmark' (△), 'Mount Barker' (+), 'Narrikup' (x), 'Rosabrook' (◇) and 'Woogenellup' (▽). Bars represent the mean standard error, r = 0.8.

Table 1 A simplified scale to assist emergence estimates of subterranean clover seedlings used at the Field Research Centre, Lincoln University, New Zealand.

Score	0	1	2	3	4	5
Number of seedlings/m^2	0	250	570	890	1200	1520

Table 2 Mean burr burial (%), number of burrs/m^2, number of seeds/burr, number of seeds/m^2 and seed yield (kg/ha) for seven subterranean clover cultivars after seed set in December 2016, at the Field Research Centre, Lincoln University, New Zealand.

Cultivar	Burr burial (%)	Burrs/m^2	Seeds/burr	Seed number/m^2	Seed yield (kg/ha)
'Antas'	40.0_{b}	2600_{b}	3.5_{a}	8700	815
'Coolamon'	75.0_{a}	3920_{ab}	2.3_{cd}	9270	570
'Denmark'	60.0_{ab}	3100_{ab}	3.0_{ab}	8845	515
'Mount Barker'	48.0_{ab}	5390_{a}	2.1_{d}	11500	970
'Narrikup'	72.0_{a}	3410_{ab}	2.8_{bc}	8710	550
'Rosabrook'	69.0_{ab}	3125_{ab}	2.2_{cd}	5725	340
'Woogenellup'	63.0_{ab}	3590_{ab}	3.0_{ab}	11120	1050
Mean	61.0	3590	2.7	9120	690
P_{value}	0.007	0.04	<0.001	0.35	0.04
MSD	29	2550	0.6	7960	725

Note: Numbers with a letter subscript in common are not significantly different (α = 0.05, THSD).

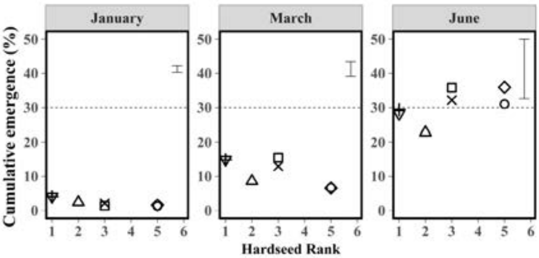

Figure 3 Mean cumulative percentage of emergence over time (January, March and June 2016) against hard seed rank (0-10, Nichols *et al.* 2013) for subterranean clover cultivars 'Antas' (□), 'Coolamon' (○), 'Denmark' (△), 'Mount Barker' (+), 'Narrikup' (x), 'Rosabrook' (◇) and 'Woogenellup' (▽). Bars are maximum standard error of the mean. Cultivar differences were: P = 0.20, MSD = 4.30 (January); P = 0.04, MSD = 11.3 (March); P = 0.90, MSD = 39.0. (June)

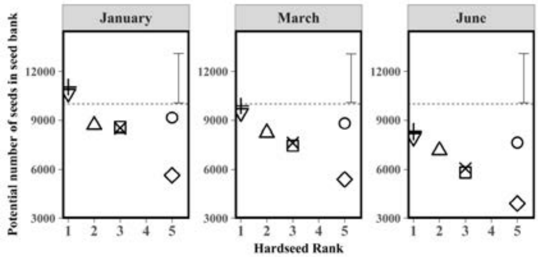

Figure 4 Mean estimated potential number of seeds (seeds/m^2) in seed bank against hard seed rank (0-10, Nichols *et al.* 2013) for subterranean clovers 'Antas' (□), 'Coolamon' (○), 'Denmark' (△)'Mount Barker' (+), 'Narrikup' (x), 'Rosabrook' (◇) and 'Woogenellup' (▽). Bars are maximum standard error of the mean. Cultivar differences were: P = 0.40, MSD = 7824 (January); P= 0.59, MSD = 7862 (March); P = 0.57, MSD = 7986 (June).

with previously reported seed weights for these cultivars (Nichols *et al.* 2013). At the first emergence in January, 'Mount Barker' and 'Woogenellup' had ~ 4% of total seeds emerged while 'Coolamon' and 'Rosabrook' had fewer than 2% (Figure 3).

By March, 'Coolamon', 'Denmark' and 'Rosabrook' had <9% of seed emerged. In June, the total cumulative percentage of seed emerged after three emergence events was 31%. Table 3 shows the actual values of seedlings/m^2 and emergence scores for each cultivar. The proportion of ungerminated seeds in January and March 2016 was positively correlated (r = 0.80 and r = 0.70, respectively) with the Australian hardseededness rankings. In June, the seedling populations for all cultivars were greater than 1000/m^2. The correlation with hardseededness rankings was r = 0.60 and about 70% of the total number of seeds remained in the soil (Figure 3). These values corresponded to ~ 8000 seeds of 'Woogenellup' and 'Mount Barker' (Figure 4).

Discussion

The lenient cut regime (mechanical mowing in late-September and mid-November) aimed at maximising seed set, was as recommended for first year subterranean clover sowing (Smetham & Dear 2003). This ensured that 'Mount Barker' and 'Woogenellup' produced >950 kg/ha and all other cultivars had seed yield above 260 kg/ha, the value considered the minimum for successful persistence (Smetham 2003). Individual cultivar yields ranged from 340 - 1000 kg/ha. The number of burrs/m^2

Table 3 Visual scores and seedling number/m^2 for seven subterranean clover cultivars measured in January, March and June 2016 at the Field Research Centre, Lincoln University, New Zealand.

	January		March		June	
Cultivar	**Score**	**Seedlings/m^2**	**Score**	**Seedlings/m^2**	**Score**	**Seedlings/m^2**
'Antas'	0.5	110	4.0_{a}	1120_{a}	5.0	1650
'Coolamon'	0.5	110	1.3_{c}	347_{c}	3.5	1150
'Denmark'	0.8	150	2.0_{bc}	450_{bc}	3.3	1070
'Mount Barker'	1.8	460	4.0_{a}	1160_{a}	4.8	1570
'Narrikup'	0.8	190	3.3_{ab}	884_{ab}	4.8	1570
'Rosabrook'	0.3	80	1.0_{c}	252_{c}	4.5	1480
'Woogenellup'	1.8	470	4.5_{a}	1200_{a}	4.5	1480
Mean	1.0	226	3.0	773	4.0	1420
P	0.07	0.05	<0.001	<0.001	0.09	0.08
MSD	1.9	483	1.7	504	2.0	687

Note: Numbers with a letter subscript in common are not significantly different (α = 0.05, THSD).

and individual seed weight were the main components that determined seed yield. These results demonstrated the high potential seed yield of all seven subterranean clover cultivars. The cumulative percentage of seed loss due to emergence in January and March was ≤15%, meaning that despite two "false strike" events, ~ 85% of the seeds potentially remained in the seed bank. After the summer rains, 'Mount Barker' and 'Woogenellup' (hardseed score = 1) had the greatest seedling emergence. With the summer rains in December and January, 'Woogenellup' and 'Mount Barker' had 4% of seeds emerged. The seedlings that emerged with the second strike in March would be expected to survive at this location in most years. However, they were sprayed and were unlikely to have survived the 2016 autumn which was one of the warmest on record with < 60% of the long-term average rainfall (NIWA 2017). Had more frequent follow-up rain kept them alive, the early establishing cultivars would have had an advantage in canopy development and pasture growth in a favourable autumn (Moot *et al*. 2003; Teixeira *et al*. 2017). 'Rosabrook' and 'Coolamon' had delayed emergence which indicates these cultivars, with hardseed ranks above 4, may not be as suitable for dryland pasture systems in New Zealand as cultivars with lower ratings.

Conclusions

- "False strike" losses did not exceed 15% of the total seeds produced, with 'Woogenellup' and 'Mount Barker' having the highest seedling losses due to early summer rains
- The Australian cultivar rankings were reliable under Lincoln, Canterbury, conditions
- Cultivars produced sufficient seed (340 -1050 kg/ha) to establish satisfactory populations in their second year despite two episodes of "false strike".

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