# An early maturing *Trifolium subterraneum* L. var. y*anninicum* with improved agronomic traits

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## Abstract

The shorter growing seasons currently being experienced in southern Australia have, in part, been attributed to the impacts of climate change. The result is an increasing requirement for pasture cultivars with earlier maturity. The aim of the current research was to develop a new cultivar of *Trifolium subterraneum* L. var*. yanninicum* (subclover) that has early maturity and improved agronomic traits over existing cultivars. In particular, early winter dry matter (DM) yield (period of lowest production), and increased tolerance to root disease. Based on historical data, three experimental lines (FS8, FS12 and FS20) were selected for evaluation against five commercial *T. subterraneum* var. *yanninicum* cultivars (Trikkala, Riverina, Gosse, Larisa and Napier). These were included in three field trials (located in South-East South Australia and in Western Victoria) from 2007-2009 inclusive. Plant characteristics deemed important were measured: days to flowering; seed yield; hardseed content; seedling regeneration; seasonal DM production; and root disease tolerance. The results revealed FS8 and FS20 to have an excellent overall performance compared to the commercial cultivars. In comparison with Trikkala (earliest maturing cultivar.), FS8 and FS20 had significantly ($P<0.05$) improved early winter DM yield (47 % and 46 % respectively) and *Phytophthora clandestina* (root disease) tolerance. FS8 flowered significantly ($P<0.05$) earlier (2 days) than FS20 and cv. Trikkala. This research has identified a new early maturing subclover with improved agronomic traits as a prospect for commercialisation in order to assist the industry in adapting to the challenges associated with shorter growing seasons.

## Keywords

Annual pasture legume; *Pythium irregulare;* waterlogging; clover scorch resistance; oestrogenic potential

## Introduction

For over a decade, the high rainfall zone of south-eastern Australia has been experiencing lower than average annual rainfall levels (Gifford et al. 1998; Trewin et al*.* 2008), coupled with increased spring daytime temperatures (B Trewin 2010, National Climate Centre, pers. comm.). The result has been a lack of soil moisture particularly towards the end of the growing season. These climatic shifts are thought by some to be broadly consistent with climatic change models, and possibly also the result of decadal natural variability (B Trewin 2010, National Climate Centre, pers. comm.). Regardless of the cause, there is an increasing demand for annual pasture legumes with earlier maturity. Early maturity confers higher seed yield potential and more reliable regeneration in short growing-season environments. Maturity can be defined as the time from sowing to when the first viable seed is produced. However, days to first flowering is easier to examine and is therefore often used as an estimate for maturity (Nichols et al. 1996), as it will be for the current experiment.

Subterranean clover (subclover) (*Trifolium subterraneum*) cultivars belonging to the variety (var.) *yanninicum* are widely sown throughout the high rainfall zone. They possess a number of desirable agronomic traits including being well adapted to waterlogging (Nichols et al. 1996). During the period 1977 to 1982, Dr. Philip Beale made a series of var. *yanninicum* crosses, principally to incorporate high levels of clover scorch resistance into a range of new cultivars (Mitchell et al. 1988). A number of elite breeding lines were produced, one of which subsequently became commercialised as the cultivar (cv.) Gosse (Oram 1992).

The aim of this experiment was to evaluate three of the earliest flowering breeding lines (FS8, FS12 and FS20) from Dr. Beale's breeding work, with the objective of selecting an early maturing cultivar with improved agronomic traits over existing cultivars. Early winter dry matter (DM) yield is usually a period of critical feed shortage and consequently is a particularly valuable trait. It has been estimated that every 1 kg of DM produced in autumn-winter can be valued at 10 times that grown in spring (Nichols *et al.* 1996). Consequently, increased early winter DM yield was one of the principal characteristics sought in this study. Lines with increased tolerance to the root diseases *Phytophthora clandestina* and *Pythium irregulare,* currently considered to be two of the most agriculturally significant species, were also seen as desirable. Trikkala is presently the earliest flowering var. *yanninicum* cultivar and consequently was the primary comparator against which the breeding lines were assessed.

## Materials and Methods

*Site and trial details*

Three experimental subclover lines (FS8, FS12, FS20) and five cultivars (Trikkala, Riverina, Gosse, Larisa and Napier) were sown at two field sites in the Kybybolite (Sites 1 and 2) district of South Australia (36?55'S; 140?55'E) and one site at Benayeo (Site 3), Western Victoria (36?54'S; 141? 02'E) in June 2007. Average rainfall across the three sites was approximately 513 mm (2007), 424 mm (2008), and 487 mm (2009) (R Hawker 2010, pers. comm.). Soils at Sites 1 and 3 consisted of sandy loams, while the soil at Site 2 was a clay loam. Site 1 was irrigated during October 2007.

Entries were sown in plots measuring 2 m x 3 m, with four replicates laid out in a randomised block design. Plots were hand-sown with 20 kg/ha of inoculated, lime-pelleted seed. Red-legged earth mites and annual grasses were controlled as required. Fertiliser was applied as a 2:1 superphosphate:potash and trace elements mix at the rate of 150 kg/ha to each site in winter every year.

*Measurements*

Assessments of DM took place during the following periods: early winter (13 July – 5 August); late winter (27 August – 15 September); and spring (6 October – 30 October). DM was assessed during the early and late winter periods using a calibrated rising plate meter. Plots were subsequently mown to a height of approximately 4 cm after each assessment (with the exception of spring assessments). Assessments for DM during spring were made by cutting one 0.5 m? quadrat to ground level with a shearing piece. Excess dry herbage was raked from the plots over summer.

Flowering date was determined in 2009 by recording the date the first ten plants of each entry produced their earliest flower. Each entry was sown in a 2 m row with three replications. This trial was also located at Site 1. Seed yield was determined by sampling one 0.5 m? quadrat per plot at Site 1 in January 2008. Seed was collected using a vacuum harvester. Hardseed level was determined by placing four replicates of 100 seeds from each entry into mesh bags. These bags were maintained outdoors from January until May 2008 when they were counted after seven days on moistened filter paper. Seedling regeneration was assessed by counting 20 (10 cm x 10 cm) quadrats per plot at Site 1 in May 2008. *Phytophthora clandestina* and *Pythium irregulare* tolerance were assessed during 2009 in a glasshouse, using a technique described by M Barbetti (2009, pers. comm.).

*Statistical analyses*

All field data was analysed by Genstat (12$^{th}$ Edition) (Payne et al. 2009). Seedling regeneration and DM yield data were analysed spatially as linear mixed models ($P<0.05$). All other results were analysed using a general analysis of variance ($P<0.05$).

## Results

*Days to flowering; seed yield; hardseed %; seedling regeneration*

FS8 flowered two days earlier (P<0.05) than cv. Trikkala (Table 1). FS8 produced more seed (P<0.05) than FS12, FS20 and the five cultivars except cv. Trikkala (Table 1). Similar hardseed levels were found in FS8, FS20 and cv. Trikkala (Table 1). While there was no significant difference in the seedling regeneration of FS8, FS20, and cv. Trikkala, they all had higher levels of (P<0.05) seedling regeneration than the other entries (Table 1).

*DM yields*

FS8 yielded more (P<0.05) early winter DM than cv. Trikkala in all four measurements (Table 2). FS20 yielded more (P<0.05) early winter DM than cv. Trikkala in three assessments, while FS12 only outyielded (P<0.05) cv. Trikkala on one measurement (Table 2). An average of the early winter DM data revealed FS8 and FS20 had improved DM yields of 47 % and 46 %, respectively, compared with cv. Trikkala (Table 2). The late winter and spring DM yield data showed few significant differences between the experimental FS lines and the subclover cultivars (data not presented). The average total DM yield of FS8 and FS20 were both improved by 9 % compared with cv. Trikkala across the growing season (data not presented).

*Root disease tolerance*

Improved (P<0.001) tolerance to root disease caused by *P. clandestina* was found in the three experimental lines (FS8, FS12, FS20) in comparison with cv. Trikkala. No differences were found between entries in their tolerance to *P. irregulare* (Table 3).

**Table 1. Agronomic Evaluations at Site 1 (2008-2009)**

| Entry | Days to Flowering 2009 | Seed Yield (kg/ha) | Hardseed (%) | Seedling Regeneration (plants/$m^2$) |
|---|---|---|---|---|
| | | January 2008 | May 2008 | May 2008 |
| FS8 | 106 | 1990 | 68 | 28 |
| FS12 | 110 | 1726 | 82 | 18 |
| FS20 | 108 | 1665 | 63 | 27 |
| Trikkala | 108 | 2103 | 65 | 26 |
| Riverina | 113 | 1757 | 87 | 9 |
| Gosse | 115 | 1553 | 64 | 15 |
| Larisa | 122 | 1467 | 74 | 13 |
| Napier | 124 | 1222 | 85 | 4 |

| | | | | |
|---|---|---|---|---|
| L.S.D. (P<0.05) | 1 | 230 | 11 | 6 |

**Table 2. Early Winter DM Yield (kg/ha) (2008-2009)**

| Entry | Site 1 | Site 3 | Site 2 | Site 3 | Mean |
|---|---|---|---|---|---|
| | 14 July 2008 | 24 July 2008 | 25 July 2008 | 4 August 2009 | |
| FS8 | 1554 | 838 | 1196 | 1411 | 1250 |
| FS12 | 983 | 576 | 1314 | 1082 | 989 |
| FS20 | 1430 | 758 | 1393 | 1370 | 1238 |
| Trikkala | 1106 | 501 | 736 | 1056 | 850 |
| Riverina | 484 | 367 | 893 | 987 | 683 |
| Gosse | 1047 | 661 | 1110 | 1211 | 1007 |
| Larisa | 512 | 355 | 662 | 916 | 611 |
| Napier | 283 | 364 | 778 | 906 | 583 |
| L.S.D. (P<0.05) | 162 | 150 | 289 | 318 | Not applicable |

**Table 3. Severity of Root Disease caused by *Phytophthora clandestina and Pythium irregulare* (2009)**

| Entry | Severity of disease caused by *P. clandestina* at 0.5% inoculum (0-5 scale) | Severity of disease caused by *P. irregulare* at 0.5% inoculum (1-3 scale) |
|---|---|---|
| FS8 | 0.9 | 2.8 |
| FS12 | 2.0 | 2.7 |
| FS20 | 1.1 | 2.6 |
| Trikkala | 2.6 | 2.7 |

| | | |
|---|---|---|
| Riverina | 0.6 | 3.0 |
| Gosse | 1.1 | 2.6 |
| Larisa | 2.3 | 3.0 |
| Napier | 0.6 | 2.8 |
| Sig level | P<0.001 | P=0.06 |
| L.S.D. | 0.4 | Not applicable |

Severity of disease: 1 = low incidence of disease.

5 = high incidence of disease.

**Discussion**

The finding that FS8 flowers earlier than Trikkala is in line with previous data. Historical data found FS8 to mature an average of 5 days earlier than cv. Trikkala (Mitchell 1990), which contrasts with the 2 days earlier maturity found in the current study. This discrepancy is likely to be due to the affect of factors such as climate and location on the resulting flowering date (Nichols et al. 1996). FS20 and FS12 did not flower earlier than Trikkala. Further testing will confirm the relative flowering dates of FS8 and FS20.

Seed yield is an important agronomic trait relating to persistence (Nichols et al. 1996). No differences were found between FS8 and cv. Trikkala on this measure; however both entries outperform the other experimental lines and cultivars. Hardseed level is another characteristic which is closely linked to persistence (Nichols et al. 1996), and to regeneration. This is illustrated in Table 1. With the exception of Gosse, the entries with the lowest hardseed levels (FS8, FS20 and cv. Trikkala) had the highest seedling regeneration scores. This result reflects the necessity to select cultivars with hardseed levels appropriate for the environments in which they will be utilised. While high hardseed levels confer excellent long-term persistence, they can negatively impact on seedling regeneration. The poor regeneration of cv. Riverina in the present study would appear to be a good case in point.

Gains in early winter DM production are particularly valuable as it is a period of critical feed shortage. FS8 and FS20 have both demonstrated excellent potential in this regard. Minimal differences in late winter and spring DM yields were found across the entries, most likely due to compensatory gains made during the season (Nichols et al. 1996). These gains in winter productivity are profound, and reflect the significant commercial potential of these breeding lines. It should be noted that FS8, FS12, and FS20 have previously been found to have low oestrogen levels and good clover scorch resistance (Mitchell *et al.* 1988), which further contribute to their agronomic value.

The three experimental lines were found to have improved tolerance to root disease caused by *P. clandestina* compared with cv. Trikkala. This is an important outcome given the waterlogged environments that var. *yanninicum* cultivars are typically grown in. *P. clandestina* is a very significant root disease in southern Australian pastures and improvements, such as displayed by FS8 and FS20, can only enhance their performance in the field.

**Conclusions**

Evaluation of three early maturing subclover breeding lines has identified two high-performing lines in FS8 and FS20. In comparison to cv. Trikkala, both FS8 and FS20 produced excellent early winter DM yields and possessed improved tolerance to *P. clandestina*. FS8 was the only experimental line that flowered earlier than existing cultivars. In relation to the aims of the current experiment, FS8 outperformed FS20 through a higher seed yield and earlier flowering date. The early maturity and improved agronomic traits of FS8 makes it an excellent commercialisation prospect. It would provide the agricultural industry with a cultivar that is better adapted to the new climatic environment, resulting in improved production and persistence in areas experiencing shorter growing seasons.

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