Nitrogen fixation by subterranean clover in southern Western Australia

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Abstract
Subterranean clover may be under stress as a result of current cropping systems leading to sub-optimal nitrogen fixation. The aim of the study was to survey farmers' paddocks to examine the health of the nitrogen fixation process. Subterranean clover from twenty one pasture paddocks, with a range of management histories, were sampled for assessment of nodule numbers, rhizobial strain in sampled nodules, pasture composition and nitrogen fixation rate. The results showed that the current rhizobial strain was the most common strain in pasture paddocks. The amount of nitrogen fixed was above industry standards. For each tonne of legume biomass there was 20 kg of nitrogen fixed in the shoots per ha. The major factors limiting nitrogen fixation were legume plant density and legume composition of the pasture. Rhizobial strain occupying the nodules and the percentage of nitrogen fixed had relatively small impacts on the amount of nitrogen fixed. The conclusion is that subterranean clover in in this part of Western Australia appears to be in a healthy state fixing sufficient nitrogen to support crop production.

Keywords
Nnodule numbers, nitrogen fixation, rhizobial strain.

Introduction
Subterranean clover (*Trifolium subterranean* L.) has remained the dominant legume in Western Australia even though there are many other pasture species available. The study by Sanford et al. (1993) of more than 184 sites in southern WA showed that the percentage of subterranean clover nitrogen derived from nitrogen fixation (%Ndfa) averaged 72% but ranged from 0 to 100%. Since that time, subterranean clover may be under greater stress as a result of current cropping systems leading to sub-optimal nitrogen fixation. These stresses include greater frequency of crops, the greater use of herbicides, increasing soil acidity and infrequent use of the latest rhizobial strain. The aim of the study conducted with support from Grain and Graze III and DAFWA was to survey farmer's paddocks to examine the health of the nitrogen fixation process and to relate that to management and soil fertility factors at the sites surveyed.

Methods
Twenty one farmers, paddocks from 6 different farm businesses were sampled in 2016 in southern part of Western Australia. The sites were north and north-west of Mt Barker in the West Kendenup (18 paddocks) and Cranbrook localities (3 paddocks). The paddocks were selected to obtain diversity in rotation history, herbicide use (particularly sulphonyl ureas and Lontrel®), years since last cropped, re-sowing history and inoculation history. The paddocks were visited twice with the following measurements taken:

- Visual pasture composition, and biomass in late winter/early spring and late spring (20 measurements across the paddock)
- Subterranean clover nodule number using a visual rating system (1-7 rating scale from low to high – Ron Yates *pers comm*) in late winter/early spring (10 measurements across the paddock)
- Subterranean clover nodules for rhizobia identification using the MALDI-ID (www.maldiid.com, Ziegler et al. 2012) technique (in late winter/early spring, 2–3 nodules per paddock)
- Soil samples (0–10 cm) for chemical analysis in late winter/early spring (20 samples across the paddock)
- Subterranean clover and ryegrass shoots sampled in late spring for nitrogen fixation measurement using the ^{15}N natural abundance (Sanford et al. 1993) technique (two samples of each species, each sample consisting of 10 sub-samples across the paddock)

The total pasture biomass for each paddock was estimated using Pasture from Space® Plus (https://pfs.landgate.wa.gov.au/).

Results

Rhizobial strain

The current strain, WSM1325 was found in 76% of the 21 paddocks sampled (Table 1), including those paddocks that had a mix of strains. The current strain was released commercially in 2005 although it had been identified in subterranean clover pastures in the 1980s. The current strain was identified in paddocks that had been recently inoculated but also in paddocks that had never been re-sown or inoculated. Herbicides used in the crop phase did not appear to have a major impact on the occurrence of WSM1325. These results indicate that in general rhizobial strains identified as WSM1325 readily displaces older less effective strains and readily spread via seed or soil movement (machinery, vehicles, livestock and dust). The strain(s) is/are also tolerant to a range of stresses commonly found in modern cropping systems particularly herbicides. However, there were situations where WSM1325 did not displace older strains and the reason for this is not obvious. The next most common strain was CC275e (Table 1) which was found in 29% of paddocks (including mixed situations). This strain was used commercially between 1974 and 1983. One paddock (Bush north) had an even older strain used commercially between 1961 and 1970 and one paddock (Stack yard) had an exotic strain which had never been released.

Table 1. Paddock history and rhizobia strain.

Paddock name	Year inoculated	Consecutive years in pasture[A]	Rotation (P pasture. C crop)	Herbicide use in most recent crop phase[B]	Rhizobia
HM4	2016	1	1 P:2 C	1 L, 1 SU	Current (WSM1325)
HM9B	2014	3	2-3 P:1 C	none	Old (CC275e)
Old house	1960	>50	Long term P	none	Current (WSM1325)
Cottage	1960s	1	2 P:4 C	2 L, 2 SU	Current & Old (CC275e)
Fat lamb	1960s	>50	Long term P	none	Current (WSM1325)
Across the creek	2016	1	4-6 P:6 C	3 L, 3 SU	Current (WSM1325)
NNMH	1960s	>50	Long term P	none	Current (WSM1325)
Mid-west three	<2000[C]	5	5 P:3-4 C	2 SU	Current (WSM1325)
Twin sister	<2000[C]	2	3-5 P:5 C	2 SU	Current (WSM1325)
Bush north	2011	6	6 P:6 C	3 L, 3 SU	Current & Old (SRDI565)
South	2011	6	5-6 P: 6 C 6	3 L, 3 SU	Current (WSM1325)
Park clearing	2006	10	10 P: 1 C	none	Current (WSM1325)
East	2015	2	5-6 P: 6 C 6	3 L, 3 SU	Current (WSM1325)
New dam	2016	1	5-6 P: 6 C 6	3 L, 3 SU	Current (WSM1325)
New dam2	<2002[C]	1	1 P: 2 C	1 L	Current (WSM1325)
House two	<2002[C]	1	1 P: 2 C	1 L	Current (WSM1325)
North river	<2009[C]	6	6 P: 2 C	1 L	Old (CC275e)
Stack yard	2005	20	Long term P	none	Other (WSM2297)
Fern swamp	<2006[C]	1	7 P: 1 C	2 SU	Current & Old (CC275e)
House paddock	<2006[C]	9	Long term P	none	Old (CC275e)
Middle	<2006[C]	4	7 P: 1 C	none	Old (CC275e)

[A] Includes 2016, [B] L = Lontrel, SU = Sulphonyl Urea, [C] < inoculation occurred before the date shown, history of these paddocks were not known because they were farms that had been purchased by the current owners

Nodule number and nitrogen fixation

The nodule rating averaged 5.1 (1 to 7, low to high) equating to a moderate number of nodules across the 21 paddocks in late winter (Table 2). The range was from 4.1 (scarce) to 6.1 (adequate). The number of nodules was negatively related to inorganic nitrogen levels, $r^2 = 0.18$ ($P = 0.05$) but not related to soil pH (Table 2). However, one of the lowest nodule ratings occurred at the lowest pH (Middle). The nodule rating of the paddocks with the current rhizobia strain averaged 5.2 (13 sites), the paddocks with a mix of old and current rhizobia averaged 4.9 (3 sites) and the paddocks with the old rhizobia strain averaged 4.8 (5 sites). Other factors that potentially affected the nodule rating for some paddocks include waterlogging (Fat lamb) and root disease (Mid-west three). The paddock history including the amount of herbicides used in the past did not appear to affect the number of nodules (Tables 1 and 2).

The biomass nitrogen percentage across the 21 sites averaged 2.6% with a range of 2.0 to 3.4%. This was lower than and a lower range than found in the literature (average 3.2%, range 2.0-4.3% across 74 sites, Unkovich et al. 2010).The %Ndfa averaged 93% (excluding four sites with unacceptable error) with a range 81–100% (Table 2). The average was higher and with a lower range than found in the literature (average 81%, range 29–100% across 74 sites, Unkovich et al. 2010). The %Ndfa was not related to nodule number or soil inorganic N measured earlier in the growing season. Soil nitrogen at peak biomass was likely to be low because of uptake by the non-legume components in the pasture. The amount of shoot nitrogen fixed averaged 139 kg/ha with a range 79–221 kg/ha (Table 2). This was also higher than in the literature (cf. 65 kg/ha) and with a lower range (cf. 5–174 kg/ha). The amount of shoot nitrogen fixed was related to the amount of legume biomass (Figure 1). One tonne of legume biomass resulted in 20 kg of nitrogen fixed in the shoots (Figure 1) which is consistent with Unkovich et al. (2010).

The legume biomass was influenced by legume composition and the total pasture biomass. Some sites had low composition (Table 2) due to low density (first year pasture after crop) and competition from other species. The total pasture biomass was also influenced by paddock characteristics such as fertility and physical (soil texture) properties.

Table 2. Paddock soil chemical analysis and legume nodule numbers, biomass and nitrogen fixation measurements.

Paddock name	pH ($CaCl_2$)	Soil inorganic N (ppm)	Nodule No.	%Ndfa	Legume % in pasture	Legume biomass t/ha	Legume N fixed kg/ha
HM4	5.2	55	5.3	93	77	6.1	143
HM9B	4.8	52	4.8	100	64	5.5	177
Old house	4.7	40	5.1	82	62	5.9	144
Cottage	5.1	22	4.8	98	73	6.9	155
Fat lamb	5.3	30	4.1	81	74	8.6	197
Across the creek	5	10	4.8	97	41	4.2	137
NNMH	5.1	36	4.8	100[A]	61	4.2	108
Mid-west three	5	46	4.1	93	59	6.6	165
Twin sister	5.7	15	5.5	100	63	6.1	151
Bush north	5	28	4.9	89	45	5.0	139
South	4.8	22	5.8	100[A]	80	8.3	221
Park clearing	4.9	19	6.1	100[A]	40	3.6	100
East	5.3	42	5.1	100[A]	72	7.8	185
New dam	5.6	14	5.8	100	46	3.9	79
New dam2	5.3	18	5.7	93	72	5.3	112
House two	5.1	26	5.9	98	77	5.4	106
North river	5.2	18	4.9	100	51	3.2	83
Stack yard	4.9	22	5.1	85	40	4.4	115
Fern swamp	4.8	32	4.9	90	60	6.1	111
House paddock	4.6	24	5.2	89	50	5.8	115
Middle	4.3	40	4.2	99	61	6.6	181

[A] Due to errors in the technique, %Ndfa was estimated at >100%, and was therefore assumed to be 100%. These sites were excluded from statistical analysis.

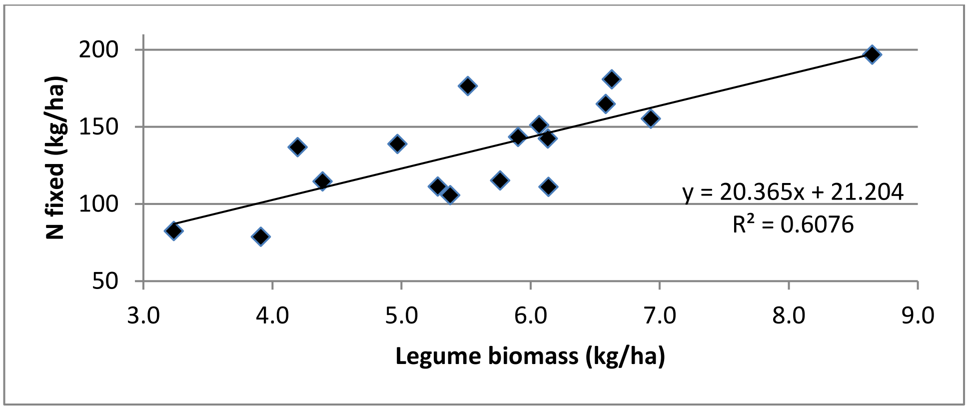

Figure 1. The relationship between the legume shoot biomass and the amount of shoot nitrogen fixed for 17 paddocks (four paddocks were excluded from analysis, see footnote Table 2).

Conclusion

Nitrogen fixation by subterranean clover in the West Kendenup and Cranbrook localities of southern Western Australia appears to be in a healthy state with adequate nodule numbers and above average %Ndfa values, relative to previous studies. This held across a range of pasture histories, from recently inoculated to paddocks inoculated 50 years ago, to paddocks that have been cropped intensively (including high use of herbicides) to those with long phases of pasture and minimal use of herbicides. In most paddocks, WSM1325, the current inoculant strain for subterranean clover, was present in sampled nodules, even in paddocks that had not been inoculated with this strain. This may indicate movement of rhizobia via animals, vehicles and machinery. Older strains of rhizobia did not appear to be a major limiting factor for nitrogen fixation. However, rainfall in 2016 was above average, the sampled paddocks are in a favourable area for agriculture and most of the soil types were sandy loams. Drier conditions may interact with other stresses on rhizobia such as long phases of crop, soil pH and herbicides to reduce the effectiveness of nitrogen fixation. Re-inoculation, where it can easily be included in management operations (such as when re-sowing), is a low cost strategy to ensure the nitrogen fixation symbiosis remains healthy.

The major limiting factor to the amount of nitrogen fixed was the amount of legume in the pasture. Improving the density of legume and reducing competition from other species would increase the amount of nitrogen fixed. If legumes are a high proportion of the pasture then this study shows that large amounts of nitrogen can be fixed in this environment in one year (>150 kg/ha). Thereafter, this nitrogen needs to be used by non-legumes in high quality pasture or as a source of nitrogen in crop production. If the nitrogen is not used there is a risk it can lead to a build-up of broad leaf or grass weeds and contribute to soil acidification if leached.

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