Identifying the causes of unreliable nitrogen fixation by strand medic (*Medicago littoralis*) based pastures

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Abstract
Following grower reports of low soil nitrogen (N) following vigorous medic-based pastures on the upper Eyre Peninsula of South Australia, management practices including herbicides and fertilisers were investigated for their effect on the N fixation of strand medic pastures at six field sites on the upper Eyre Peninsula. Applying phosphorus to soils with low P reserves at medic pasture establishment increased medic shoot dry matter and improved N fixation. The addition of urea at seeding reduced N fixation by the medics at some sites. Soil residues of the herbicide Logran reduced medic growth at one site. The application of a full label rate of Agritone 750 Herbicide (applied late when the medic plants were 5-7 cm in diameter) decreased pasture production and N fixation.

Key Words
Nitrogen fixation, pasture, herbicides, nodulation, phosphorus

Introduction
Annual medics (*Medicago spp.*) are self-regenerating legumes that are well-suited to crop rotations on neutral to alkaline soils in the low to medium rainfall areas of southern Australia. They provide highly nutritious feed for livestock, act as a disease break for many cereal root pathogens, and improve soil fertility through nitrogen (N) fixation. However, growers on the upper Eyre Peninsula (EP) of South Australia commonly report sub-optimal performance of the following cereal crops, even when the medic has been productive. Observations of poor medic nodulation and the longer term decline in grain protein levels (Wilson 2017) have led them to speculate that the pastures may not be providing sufficient fixed N. Medic pastures are often sprayed with a range of herbicides and pesticides, both to ensure the productivity of medic as pasture for livestock, and to reduce the carryover of weeds into the following cereal crop. This paper examined if commonly used management practices reduced N fixation of strand medic pastures and mineral N supply to the following crop. The work was field based and undertaken on soils typical of the upper EP. The results should also be relevant to other low rainfall systems where medics are used.

Methods
Two replicated field experiments were conducted on the upper EP in 2015, 2016 and 2017. In each year, one trial was located on a grey calcareous sandy soil on the western EP at Piednippie (32.5107S, 134.3054E: 14 mg/kg P), and the other on a red loam of central EP at Pinbong (32.5848S, 135.2238E: 16.7 mg/kg P) or Minnipa (32.5003S, 135.0903E: 14 mg/kg P). In all trials, medic was seeded at 10 kg/ha after opening rains and weed control.

In each season there were post-emergent herbicide treatments, herbicide residue treatments and nutrition treatments (Table 1). 'Early' post-emergent herbicide treatments were imposed when the medic plants reached their third trifoliate leaf stage. 'Late' herbicide treatments were applied when the medic plants were 5-7 cm in diameter. A high rate of urea was included as a treatment to simulate how medic would perform if sown into a soil with high N reserves. In the first two years, two strand medic (*Medicago littoralis*) cultivars: Herald and Angel, were used, but in 2017 only Herald was grown. Medic establishment and dry matter (DM) production, prior to flowering, were assessed and indicators of fixed nitrogen (N) production were monitored in four ways:

1. Number of viable nodules on roots 14-19 weeks after sowing;
2. Soil mineral N in the root zone the following autumn;
3. Levels of root damage using a visual score, where 0 = none and 15 = severe; and
4. N fixation by the ^{15}N natural abundance technique.

Trials were sown as a split plot design (medic variety in main plots) in 2015 and 2016, and as a completely randomised block design in 2017, with 3 replications. Trials were re-sown with wheat in the year after medic to assess the impact of treatments on subsequent cereal production. Data were analysed using Analysis of Variance in GENSTAT version 18. The least significant differences were based on F probability = 0.05.

Table 1. Selected herbicide and nutrient treatment details

Year	Treatment	Active Ingredient	Chemical Group	Application rate (units/ha)
2015, 2016	Agritone 750 Early	750 g/L MCPA (as dimethylamine salt)	I	330 ml
2015, 2016	Agritone 750 Late	750 g/L MCPA (as dimethylamine salt)	I	330 ml
2015	Logran	750 g/kg Triasulfuron	B	0.125 g
2015, 2016	Nitrogen	Urea		100 kg at sowing
2015, 2016, 2017	Phosphorus	Phosphoric acid		10 kg at sowing
2016, 2017	Tigrex Early	250 g/L MCPA as the ethylhexyl ester; 25 g/L Diflufenican	I F	100 ml + 200 ml BS1000
2016	Logran	750 g/kg Triasulfuron	B	1.25 g
2017	Agritone 750 Early	750 g/L MCPA (as the dimethylamine salt)	I	200 ml
2017	Agritone 750 Late	750 g/L MCPA (as the dimethylamine salt)	I	200 ml
2017	Logran	750 g/kg Triasulfuron	B	1.75g
2015, 2016, 2017	Control	Nil		

Results

The treatment to simulate Logran residues did not affect the growth of the group B susceptible Herald and hence Angel and Herald grew similarly. Under the seasonal conditions which prevailed in those trials, the level of Logran applied was not toxic to medic. As there were only subtle differences in the performance of Herald and its replacement group B herbicide tolerant cultivar Angel, results (Table 2, p. 3) are presented as the average of both cultivars.

Plant establishment densities ranged from 97 plants/m^2 (Pinbong in 2016) to 223 plants/m^2 (Minnipa in 2017) and were not significantly affected by the herbicide or nutritional treatment at any site. Levels of root damage were reasonably low, averaging 4.9/15 at Piednippie, 6.4/15 at Pinbong and 4.5 at Minnipa, and did not differ between treatments.

The number of nodules per plant averaged 6.5 across all treatments, sites and years. The application of Agritone 750 increased the number of nodules classified as ineffective and when applied later in the season, decreased the amount of N fixed (at Piednippie in 2015, 2016 and at Pinbong in 2016) and also decreased medic dry matter production. Logran residues did not affect nodulation, but at Minnipa in 2017, the impact of Logran on the seedlings was so severe that none were available for assessment. Logran reduced DM production from 446 kg/ha in the Control, to only 34 kg/ha. Tigrex herbicide and P application did not affect nodulation. Urea increased the proportion of ineffective nodules at Piednippie in 2015, but otherwise did not significantly affect nodulation.

P application at 10 kg/ha generally increased medic dry matter production and N fixation. For example, medic DM production was increased from 0.52 t/ha to 1.3 t/ha at Piednippie (2015), from 0.96 t/ha to 1.20 t/ha at Pinbong (2015), from 1.08 t/ha to 1.59 t/ha at Piednippie (2016), and from 0.13 t/ha to 0.28 t/ha at Piednippie (2017). Urea decreased the amount of N fixed by 16.7 kg/ha (Pinbong 2016) and 11.5 kg/ha (Piednippie 2016), but increased it by 5.3 kg/ha (Piednippie in 2015).

In 2016, 2017 and 2018, soil available N (Table 2) was not affected by treatments in the 0-60 cm zones at any of the trial sites. In both 2016 and 2017, the previous year's medic treatments had no effect on the production of the wheat grain yield or protein levels at any of the trial sites.

Discussion

N contributions from medic pastures are likely being reduced by the application of herbicides commonly used for weed control in these pastures. Agritone 750 Herbicide, when applied at label rates, was shown to increase the proportion of ineffective nodules (when applied early) and substantially reduce DM production and N fixation (when applied late). One proviso to this conclusion is that many EP farmers use rates of these herbicides well below recommended label rates so impacts of these applications may be smaller in commercial situations.

Table 2. Effect of selected herbicide and nutrient treatments on the nodulation, N-fixation and dry matter production of strand medic; wheat yield the following year; and soil mineral N the following autumn. Significant differences from the control treatment within each site/year indicated by *(P<0.05) or ** (P<0.01)

Site/year	Treatment	Nodule numbers per plant (L col. = Effective and R column = ineffective)		Medic Production (t DM/ha)	N fixed (kg/ha)	Wheat yield (t/ha)	Soil Mineral N (NO_3^- and NH_4^+ 0-60 cm, kg N/ha)
Piednippie 2015	Control	3.0	0.7	0.52	9.3	1.89	81.8
Piednippie 2015	Logran residue	2.8	0.9	0.49	8.3	1.98	102.4
Piednippie 2015	Agritone 750 Early	1.8*	1.6*	0.53	10.9	2.02	88.3
Piednippie 2015	Agritone 750 Late	2.6	1.3*	0.31**	5.4**	1.87	85.2
Piednippie 2015	Phosphorus	3.6	0.8	1.30**	23.2**	1.94	83.3
Piednippie 2015	Urea	2.2*	1.4*	0.54	14.6**	2.02	113.3
Pinbong 2015	Control	6.0	0.9	0.96	21.0	1.28	90.5
Pinbong 2015	Logran residue	5.3	1.1	0.99	23.1	1.32	88.5
Pinbong 2015	Agritone 750 Early	3.8	2.5	0.61**	15.1	1.28	111.5
Pinbong 2015	Phosphorus	5.7	0.8	1.20**	26.4	1.56	94.2
Pinbong 2015	Urea	3.4	2.3	0.82	19.5	1.23	111.0
Piednippie 2016	Control	5.5	1.5	1.14	26.5	1.47	46.4
Piednippie 2016	Logran residue	5.8	1.3	1.09	26.2	1.36	45.9
Piednippie 2016	Agritone 750 Early	5.4	2.9**	0.86	20.5	1.55	49.4
Piednippie 2016	Agritone 750 Late	4.2	2.4	0.84	17.4**	1.55	50.1
Piednippie 2016	Tigrex	6.1	1.4	0.89	21.8	1.49	40.9
Piednippie 2016	Phosphorus	6.4	1.8	1.59**	38.8**	1.38	38.8
Piednippie 2016	Urea	5.2	1.2	0.70**	15.0**	1.49	61.1
Pinbong 2016	Control	3.0	5.5	0.82	23.5	1.44	20.7
Pinbong 2016	Logran residue	2.4	5.3	0.79	22.9	1.31	21.5
Pinbong 2016	Agritone 750 Early	3.3	8.6**	0.64	19.7	1.35	21.2
Pinbong 2016	Agritone 750 Late	1.2*	6.9	0.48**	13.0**	1.37	22.1
Pinbong 2016	Tigrex	2.9	6.4	0.53**	15.2**	1.37	19.3
Pinbong 2016	Phosphorus	2.8	6.5	0.91	26.2	1.55	18.9
Pinbong 2016	Urea	3.5	5.0	0.29**	6.8**	1.43	23.5
Piednippie 2017	Control	1.7	5.7	0.13	2.8	-	43.6
Piednippie 2017	Logran residue	1.6	3.5	0.14	2.9	-	53.2
Piednippie 2017	Agritone 750 Early	1.5	5.7	0.13	3.0	-	52.0
Piednippie 2017	Agritone 750 Late	1.1	5.4	0.16	3.8	-	53.2
Piednippie 2017	Tigrex	1.1	5.1	0.21	4.0	-	45.6
Piednippie 2017	Phosphorus	2.3	5.2	0.28**	6.2**	-	42.0
Minnipa 2017	Control	0.5	5.7	0.45	5.1	-	29.5
Minnipa 2017	Logran residue	-	-	0.03**	-	-	40.7
Minnipa 2107	Agritone 750 Early	0.0	5.4	0.30	2.8	-	35.5
Minnipa 2017	Agritone 750 Late	0.4	6.0	0.43	5.3	-	21.7
Minnipa 2017	Tigrex	0.3	6.3	0.36	3.7	-	29.5
Minnipa 2017	Phosphorus	0.9	5.3	0.43	4.2	-	32.9

The significant benefits of P applications to medic growth and N fixation reinforce the value of sound P nutrition to optimise medic performance. Nodule number was less than 12 per plant, which is less than for other legume species (Drew et al. 2012). This low number of nodules on strand medic is often interpreted as

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being insufficient, but is probably reasonable for the species, noting that the average amount of N fixed/t shoot DM in the trials was 22 kg and similar to that for other pasture legumes (Unkovich et al. 2010).
In general, biomass production and total N contribution from the sown medic pastures was low in the newly established medic pastures, and likely explains why no significant differences in soil mineral N were measured in the years following the medic pasture. In regenerating medic pasture, treatment impacts on medic growth and N fixation could be greater because they usually produce more biomass than seeded stands, and therefore are likely to have greater impacts on the following cereal crop.

Conclusions

These trials have shown that applying P when establishing medic pastures can substantially increase their productivity, whereas using certain herbicides can significantly damage medics by reducing their ability to grow, maintain effective nodules and/or fix nitrogen. Herbicides are an essential part of weed management, but their negative effects on medic pasture growth for N production and livestock feed must be considered from a whole farming systems perspective rather than solely to the value of the weed control they provide.

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