Nodules or not – a survey of pasture legume nodulation in central and southern NSW

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Abstract
Two hundred and twenty-five paddocks were sampled across the central and southern regions of NSW to quantify the nodulation status of pasture legumes in existing pasture paddocks. Results of this survey found that 90% or more of all paddocks had inadequate nodulation and in some regions up to 20% of sampled paddocks had no nodules present on plants sampled. Based on soil pH levels, clover and medic micro-symbionts would be expected to perform sub-optimally in more than 70 and 90% of sampled paddocks respectively. Inadequate levels of key plant nutrients, particularly sulphur, were prevalent across all regions. The results of the survey indicate that the provision of nitrogen via biological nitrogen fixation may be limited and considerably less than that commonly reported in the literature. It appears there is a lack of awareness of this issue within the industry and determining nodulation of representative plant roots carefully excavated from the soil is a first and critical step in assessing nitrogen fixation potential of a legume-based pasture paddock. Addressing the issue of poor nodulation is likely to require amelioration of widespread soil acidity issues and provision of soil nutrients essential for symbiotic nitrogen fixation.

Keywords
Rhizobia, pasture legume, nodulation, pH, sulphur, phosphorus.

Introduction
The role of legumes in fixing nitrogen and the benefit of nitrogen fixation for growth of non-leguminous pasture species or following crops has long been recognised. Pasture legumes that are adequately nodulated by appropriate rhizobia are generally quoted as fixing 20-40 kg N/t DM produced (Howieson et al. 2000). The estimates of nitrogen fixation are frequently made without assessment of the nodulation status of the legumes. In the mixed farming zone, the primary role of pastures is to increase nitrogen supply for the next cropping phase, as well as feed for livestock. Similarly, in the permanent pasture zone, legumes are presumed to supply nitrogen for non-leguminous pasture species thus increasing overall pasture growth and livestock production while being a valuable feed source in their own right. Given returns from cropping enterprises have been low recently, the role of legumes in fixing nitrogen is becoming more critical to reduce reliance on fertiliser N sources and improve profitability of the cropping phase. Additionally, livestock prices are relatively high and recent surveys indicate more than 95% of producers will be either maintaining or increasing the role of livestock enterprises in the overall farming system (Hackney et al. 2017) therefore the importance of legumes in fixing nitrogen for sustaining these enterprises is critical. The purpose of this survey was to assess nodulation in existing pasture paddocks throughout central and southern NSW to ascertain whether pastures are currently likely to be fulfilling nitrogen supply expectations and to identify possible soil factors contributing to nodulation success or failure.

Methods
Four regions (Central Tablelands, Central West, Monaro and Riverina) were included in this survey. Sampling occurred in late winter-early spring of 2015 for Central West (n=60 paddocks) and Riverina (n=81) and in 2016 in spring for Central Tablelands (n=30) and Monaro (n=54) regions. All paddocks sampled were on commercial farms. Paddocks were selected by expression of interest and through contacts in farming system groups and retail agronomist/consultant networks. Paddocks sampled were those

volunteered by the property owner with the only stipulation being they must contain some legume component. A 20 m x 20 m sampling area was established in a representative area of the paddock. A minimum of 15 legumes of each dominant species were carefully excavated and the root systems gently washed. Root systems in fine textured soils were soaked to allow passive and intact exhumation. Nodulation on each individual plant was scored using the 0-8 scoring system of Yates et al. (2016); where a score of 4 is considered adequate nodulation. Soil samples (0-10 cm) were collected from within the sampling area for chemical analysis. In addition, botanical composition was assessed using the rod-point method (80 points) around the perimeter of the sampling area and across the diagonals). The GPS location of the sampling area was taken from the intersection of the diagonals. Producers also provided information on paddock history (pasture age, fertiliser and chemical history, method of rhizobia delivery) which will be presented in future publications.

Results

Legume abundance in paddocks sampled varied considerably within and between regions with the Central West and Riverina having higher levels than other regions. Pasture legume species sampled in the survey were predominately subterranean clover (*Trifolium subterraneum*), while annual medics (*Medicago* spp.) were present in at least one-third of paddocks sampled in the Central West. This is to be expected given the long history of use of these species across the survey regions. Naturalised legumes including haresfoot clover (*T. arvense*) and suckling clover (*T.dubium*) were found paddocks in the Central Tablelands and Monaro region. While species such as arrowleaf clover (*T. vesiculosum*), bladder clover (*T. spumosum*) and biserrula (*Biserrula pelecinus*) were not encountered frequently in the survey, where they were recorded, it was generally in high abundance, particularly in the Central West (>65% when present). Similarly, the perennial legumes Caucasian clover (*T. ambiguum*) and red clover (*T. pratense*) were not encountered frequently in the Monaro region, but where found, they singularly or in combination accounted for up to 55% of legume frequency recordings. White clover (*T. repens*) was found at varying frequencies in the Central Tablelands and Monaro regions and lucerne (*M. sativa*) was encountered at varying densities across all regions. It should be noted that lucerne was only sampled where it had been sown in the survey year due to the difficulty in excavating older, established lucerne plants.

Table 1. Legume nodulation, legume content (%) and selected soil chemical parameters from a legume nodulation survey of 225 Paddocks across the Central Tableland, Central West, Monaro and Riverina regions of NSW. Range of results in parentheses. [1]For nodulation shows percentage of paddocks with nodulation score <4. pH first number is percentage of sampled paddocks with pHCa<5.5 and in parentheses pHCa<7.0. For exchangeable aluminium <5% of CEC, the level above which lucerne likely to be sensitive. Colwell P paddocks with less than critical soil available P based on PBI and sulphur paddocks less than 8 mg/kg (Gourley et al. 2007).

	Legume (%)	Average nodulation score (0-8)	pH_{Ca} (0-10 cm)	Al (% CEC)	Mineral N (mg/kg)	P (Colwell) (mg/kg)	S (KCl-40) (mg/kg)
Central Tablelands	27 (7.5-53)	2.3 (0.8-4.3)	5.0 (4.4-5.7)	3.1 (0.1 -12)	36 (12-66)	30 (6-133)	7.1 (2.7-14.6)
Paddocks less than critical (%)[1]		93	87 (100)	80		63	70
Central West	50 (7.5-95)	1.8 (0-6.7)	5.2 (4.3-7.6)	1.9 (0-10)	16 (1.8-99)	29 (8-150)	5.0 (0-18)
Paddocks less than critical (%)		90	73 (96)	90		41	95
Monaro	24 (4-68)	2.6 (1.1-5.1)	5.1 (4.2-8.2)	4.1 (1.0 -29)	21 (5.6-55)	44 (11-120)	7.9 (2.0-31)
Paddocks less than critical (%)		96	80 (94)	81		35	63
Riverina	52 (9-93)	2.2 (0.5-4.9)	5.2 (4.3-6.7)	3.5 (0.5-24)	22 (5.0-50)	42 (6-170)	6.5 (1.0-24)
Paddocks less than critical (%)		96	75 (100)	80		37	72

Ninety percent or more of all paddocks sampled had nodulation scores less than 4 (Table 1). In the Central West, 20% of paddocks sampled had no nodules at all. Interestingly, pasture appearance above ground was not a reliable indicator of nodulation status with plants appearing healthy in general. Poor nodulation was common across all species sampled with the exception of arrowleaf clover (Central West and Monaro), bladder clover, biserrula, red clover and Caucasian clover which had adequate or higher than adequate (score ≥4) nodulation in most instances where they were sampled. These were generally pastures that had been established for less than three years, whereas those containing subterranean clover and annual medics ranged in age from newly sown to more than 50 years old.

Average soil pH was similar across all regions but there was considerable variation within regions. In terms of rhizobial function, more than 70% and 94% of paddocks had a soil pH considered less than optimal for the survival and performance of *Rhizobium leguminosarum* bv. *trifolii* and *Sinorhiozbium medicae*, the mico-symbionts of clover and medic legumes respectively (Table 1) (Drew et al. 2014; Yates et al. 2016). Ten percent (Central West) and 20% of paddocks (all other regions) had exchangeable aluminium levels (>5%) which are likely to adversely impact on lucerne growth (Table 1) (Lattimore 2008).

Soil mineral N was highest on the Central Tablelands compared to other regions (Table 1). Assuming a typical bulk density for soils in this survey of 1.2 g/cm^3 for 0-10 cm soil depth, this equates to absolute mineral nitrogen availability of 43 kg N/ha for the Central Tablelands, 20 kg N/ha for Central West and 25 kg N/ha for both the Monaro and Riverina respectively. Nitrate accounted for 60%, 78%, 37% and 35% of mineral N in the Central Tablelands, Central West, Monaro and Riverina regions respectively. Low levels of available phosphorus were encountered more frequently in the Central Tablelands than in other regions with more than 60% of paddocks having a Colwell P less than the critical value (Table 1). Sulphur deficiency was prevalent across all regions (more than 60% paddocks deficient across all regions) but was particularly severe in the Central West where all but three of the paddocks sampled had inadequate sulphur (Table 1).

Conclusion

Inadequate nodulation in more than 90% of the 225 pasture paddocks sampled in this survey is cause for significant concern. Given these results it would appear unlikely that pasture legumes are fixing nitrogen in quantities suggested in the literature. It is particularly worrying that up to 20% of paddocks in some regions have no evidence of nodulation whatsoever. Provision of nitrogen to non-legume plants (or legumes that have failed to nodulate) can only come from the soil nitrogen pool. In essence, the results of this survey show that pasture legumes are potentially satisfying a large proportion of their nitrogen needs from this pool rather than increasing organic nitrogen via nitrogen fixation. High levels of mineral N can suppress nodulation (both nodules number per plant and nodule mass) as well as nitrogen fixation (Unkovich et al. 1996). Unkovich et al. (1996) in discussion, describes soil mineral nitrogen exceeding 40 mg/kg, recorded in 5 t/ha lime application treatments in a Western Australian field study as sufficient to reduce nodulation in subterranean clover. Therefore, in the current survey, it is unlikely that mineral nitrogen was supressing nodulation, particularly in the Central West, Riverina and Monaro regions (16-22 mg N/kg). However, soil mineral nitrogen levels approached this threshold (36 mg N/kg) on the Central Tablelands. Similarly, the conceptual model of Denton et al. (2011) indicates where soil nitrate levels exceed 10 mg/kg, reduction in both nodulation and nitrogen fixation may be expected. Using this benchmark, it is again unlikely that there has been significant impact of soil nitrate on nodulation in either the Monaro or Riverina (both average 8 mg/kg). It is possible that minor effects on nodulation may have occurred due to nitrate levels in the Central West (12 mg/kg) while reduction in nodulation in the Central Tablelands may be indicated due to an average soil nitrate level of 20 mg/kg. Caution is required in interpreting the impact of soil mineral nitrogen from one point in time. The mineral nitrogen value depends on the relative rate of mineralisation from soil organic matter compared to the uptake by plants and therefore it is not possible to determine if soil nitrogen supply and plant demand are in synchronicity at a given point in time.

Plant tolerance to soil pH is well documented, and commonly encountered in scientific and extension publications. However, there appears to be less appreciation of the impact soil pH has on rhizobial function. This survey has demonstrated that a high proportion of paddocks sampled have a soil pH that is sub-optimal for the function of clover and medic micro-symbionts (Drew et al. 2016). Increasing nodulation through amelioration of acidic soils with lime has been documented by a number of authors over time (e.g. Richardson et al. 1988). Similarly, Unkovich et al. (1996) reported increases in the proportion of nitrogen

derived from the atmosphere in legumes growing in lime amended soils. The consequence of this poor fixation is that the legume relies on the soil nitrogen pool for its nitrogen needs rather than fixed N.

Low soil sulphur levels were also very commonly encountered in this survey and this appears to be a more common deficiency than phosphorus across the survey regions. Though not presented here, many producers were using low sulphur cropping fertilisers such as MAP or DAP for pasture establishment and many pastures used in crop-pasture rotation systems were not receiving any fertiliser during in the pasture phase. The lack of, or infrequent use of sulphur may be an issue that requires attention given the requirement of sulphur for protein function. Scherer and Lange (1996) have shown increases in nitrogen fixation in legumes supplied with increased rates on sulphur fertiliser.

Producers depend heavily on legumes and their ability to fix nitrogen for both crop and livestock production. The findings of this survey indicate urgent need for producers and advisors to check not only that legumes are growing well, but that they are also adequately and effectively nodulated. Achieving this may require amendment of soil pH issues which are widespread across the survey regions as well as ensuring adequate provision of nutrients to enable nodulation and nitrogen fixation to occur.

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