# Reduced nodulation of faba bean on acidic soils; the role of topsoil pH stratification

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## Abstract
The performance of most pulse species on the acidic soils of south eastern Australia is spatially and seasonally variable. Our research, aiming to identify reasons for this variability, reports the detrimental effects of acidic soils on nodulation of the sensitive pulse crops, mainly faba bean. A survey of 39 commercial pulse crops, conducted across this zone in 2015 and 2016 found nodulation to be inhibited at $pH_{Ca}$ < 5.2 in the 0-10 cm layer. Follow-up investigations found acidic soil layers below 5 cm negatively impacted nodulation, root growth and vigour of faba bean crops. Moderately ($pH_{Ca}$ 4.5-5.0) and severely ($pH_{Ca}$ <4.5) acidic layers in the 5-20 cm soil profile were not detected using soil samples collected at sampling depths of 0-10 cm and 10-20 cm. Sampling at 5 cm intervals is recommended to detect pH stratification. The industry practice of spreading lime with no incorporation and sowing with minimum soil disturbance, confines the lime effect to the shallow surface layers (0-5 cm). A rapid solution to intensely stratified pH in the soil surface (0-10 cm) requires an aggressive approach including appropriate lime rates and strategic cultivation to a depth of 10 cm, at least 12 months prior to sowing sensitive species. The pH stratification presented in this paper is more intense and widespread than previously reported. Further investigation is needed to: (i) assess the effectiveness of current approaches to acid soil management, and (ii) quantify the impact of pH stratification on more acid-tolerant species including canola, cereals, lucerne and clovers).

## Keywords
Stratified pH, rhizobia, acidity, lime, incorporation.

## Introduction
While faba bean, lentil and chickpea, are generally acknowledged as being sensitive to soil acidity, they can be successfully grown on slightly acidic soils ($pH_{Ca}$ >5.2 - 6.0) in the high (HRZ) and medium rainfall zones of southern Australia, albeit with somewhat inconsistent yields. In NSW the HRZ is dominated by acidic soils (0-10 cm; $pH_{Ca}$ < 6.0). Lupin species make up 49% of the southern NSW pulse area (Richards and Gaynor 2016) and adoption of acid-sensitive species, such as faba bean, lentil and chickpea, is limited by variable yields, volatile markets, perceived high risk and poor adaptation. This paper identifies factors affecting nodulation and early vigour of pulse crops, mainly faba bean, grown on acidic soils in the HRZ of south eastern Australia.

Guidelines for tolerance of pulses to soil acidity are vague, with the ideal $pH_{Ca}$ for faba bean at 6.0-8.0, down to a minimum $pH_{Ca}$ of 5.2 (Matthews and Marcellos 2003). While the optimal $pH_{Ca}$ range for *Rhizobium* spp. used for faba bean, lentils and chickpea is > 6.0, these rhizobia species are sensitive to $pH_{Ca}$ < 5.0 (Drew et al. 2012). The main pathway for rhizobial infection of these species is via root hairs and the environment to which the rhizobia and host plant are exposed influences root development and rhizobial function and therefore the success of the complex nodulation process (Drew et al. 2012; Cregan and Scott 1998).

There is a pressing need to increase the diversity of crop options available in the acid soil HRZ as the dominant wheat/canola cropping systems are increasingly constrained by reliance on N fertiliser prices and herbicide resistance. Pulses are an attractive option but overcoming soil acidity constraints is a prerequisite. Numerous studies report the presence of acidic layers at 5-20 cm in both agricultural and non-agricultural soils (e.g. Paul et al. 2003) although such observations have rarely been extended to examine the effect of such layers on plant growth. As pulse seed is typically sown at depths of 6-8 cm, this study investigated the effect of soil acidity below 5 cm depth on nodulation and root growth of commercial faba bean crops.

## Methods
*Acid-sensitive pulse crops and soil acidity*
In 2015 and 2016, 39 commercial pulse crops were monitored in NSW, Victoria, SA and Tasmania, including faba bean, lupin, field pea, lentil and chickpea. The 2015 sites provided geographical spread across

acid soil regions of the HRZ and included 12 paddocks of faba bean. In 2016 an additional 5 growers were engaged to investigate a broader range of pulses and soil types - sodosols, chromosol and rudosols. Sites monitored in 2016 included 14 faba bean crops.

A uniform, one hectare area of crop was selected at each site with soils sampled at depths of 0-10 cm and 10-20 cm, and $pH_{Ca}$ measured. Plants were collected at random from the designated areas, 2-3 months post-emergence, then assessed and scored for effectiveness of nodulation using the Columbia protocol (Anon 1991). Scores were allocated for (1) plant growth/vigour, (2) nodule number, (3) nodule position, (4) nodule colour, and (5) nodule appearance; all parameters had equal weighting with '25' the maximum total score.

*Soil pH stratification and the effect of acidic layers on root development and nodulation of faba bean*
In 2015, crops with low nodulation scores (< 18) were investigated further. Root growth was assessed *in situ* and soil cores divided into 2.5 cm intervals to a depth of 15 cm and tested for pH (Manutec® Soil pH Test Kit). In 2016 root growth was assessed *in situ* and soil cores divided into 2.5 cm increments (to a depth of 10 cm) and 5 cm increments from 10-20 cm. Soil pH was measured in the NSW DPI Wagga Wagga laboratory using the calcium chloride method (Rayment and Higginson 1992).

## Results and Discussion

*Acid sensitive pulse crops and soil acidity*
Faba bean was the most commonly grown pulse species in this study, enabling us to identify common constraints across NSW, SA, VIC and TAS, which we propose are relevant to other acid-sensitive legume species. Analysis of the nodulation scores for faba bean crops and $pH_{Ca}$ of 0–10 cm soil samples from the monitored paddocks showed a strong correlation ($r^2 = 0.89$) between soil $pH_{Ca}$ and nodulation scores (Figure 1). The nodulation of faba bean was adversely affected at soil $pH_{Ca}$ below 5.2 for 0-10 cm, although this critical pH for 0-10 cm samples would be modified by pH stratification. The form of inoculant used (peat slurry, freeze dried or granular) did not have a significant effect on nodulation score.

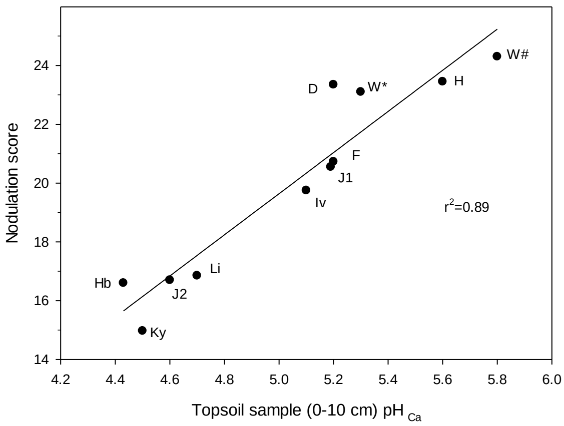


**Figure 1. The effect of surface soil $pH_{Ca}$ (0-10cm) on nodulation of faba bean at 11 sites in the SE Australian high rainfall zone. Sites monitored in 2015 included Kybybolite, SA (Ky), Holbrook, NSW (Hb), Lismore, Vic (Li), Inverleigh, Vic (Iv), Frances, SA (F), Darlington, Vic (D), Willaura, Vic (W) and Henty, NSW (H); and 2 sites at Junee, NSW (J1 and J2) monitored in 2016. W* = after wheat, W# = after canola.**

The monitored faba bean fell into two distinct categories: (i) vigorous, well nodulated crops; and (ii) those with a nodulation score below 18, which included extremely variable crops that showed symptoms of nitrogen deficiency within 3 months of emergence. This was most evident at the Holbrook (Hb), Kybybolite (Ky) and Junee (J2) sites, which recorded nodulation scores of 17, 15 and 16.6, respectively. Although the percentage of exchangeable aluminium at the Holbrook site was 8% in the 0-10 cm and 35% at 10-20 cm, the percentage of exchangeable aluminium at the Kybybolite site was < 2%, suggesting that low pH was the main factor that reduced nodulation in those soils, rather than aluminium level.

*Soil pH stratification and the effect of acidic layers on root development and nodulation of faba bean*
Only the 2015 faba bean crops at Holbrook, NSW (Hb), Kybybolite, SA (Ky), and the 2016 sites at Junee, NSW (J1 and J2) are discussed. All these sites, except Kybybolite, had received applications of lime within the last 5 years. Lime was applied at the Holbrook site in 2010 and again in 2015 at 2 t/ha. The Junee sites were within the same paddock and received lime at a rate of 1.13 t/ha in 2011, which was not incorporated.

Composite soil samples taken at depths of 0-10 cm and 10-20 cm, failed to detect significant variation in soil pH down the profile at Holbrook and Junee (Table 1).

Despite the Holbrook site receiving 4 t/ha of lime since 2010, incorporation with a Speedtiller® and the disc seeder sowing system was ineffective in mixing the lime below 5 cm. Finer soil sampling of the topsoil indicated that at a sowing depth of about 6-8 cm the emerging seedlings and rhizobia were exposed to a hostile environment ($pH_{Ca}$ <4.4), two pH units and 100 times more acidic than the surface soil ($pH_{Ca}$ 6.5). Nodulation and root hair density was poor and the crop was showing symptoms of acute nitrogen deficiency within 3 months of emergence. Roots did not penetrate into the severely acidic soil below 5 cm; they were stunted, thickened and distorted, all symptoms typical of aluminium toxicity.

At the Kybybolite site nodulation was poor and plant roots were also stunted, thickened and distorted. However, with exchangeable aluminium levels of <2%, it is possible that severe acidity ($pH_{Ca}$ <4.5) may have been sufficient to restrict root growth and disrupt the nodulation process (Cregan and Scott 1998).

**Table 1. $pH_{Ca}$ measurements of 0-10 cm and 10-20 cm soil profiles fail to detect the pH stratification at the Holbrook, Kybybolite and Junee sites, compared with tests from smaller sampling increments.**

| Soil depth (cm) | Holbrook site (Hb) | | Kybybolite site (Ky) | | Junee 1 site (J1) | | Junee 2 site (J2) | |
|---|---|---|---|---|---|---|---|---|
| | Soil $pH_{Ca}$ | | Soil $pH_{Ca}$ | | Soil $pH_{Ca}$ | | Soil $pH_{Ca}$ | |
| | Nodulation score – 17 | | Nodulation score - 15 | | Nodulation score – 20.6 | | Nodulation score – 16.6 | |
| | Composite sample | Sub samples* | Composite sample | Sub samples* | Composite sample | Sub samples | Composite sample | Sub samples |
| 0-2.5 | 4.6 | 6.5 | | 4.2 | | 5.5 | | 4.9 |
| 2.5-5 | *(Grower's* | 5.6 | | NA | | 5.4 | | 4.6 |
| 5-7.5 | *pH test - 5.2)* | 4.4 | 4.5 | NA | 5.2 | 5.2 | 4.4 | 4.2 |
| 7.5-10 | | 4.2 | | 4.0 | | 4.6 | | 4.1 |
| 10-15 | | 4.1 | | NA | | 4.6 | | 4.2 |
| 15-20 | 4.1 | 4.1 | 5.7 | NA | 4.8 | 5.0 | 4.4 | 4.6 |

* $pH_{Ca}$ for Holbrook and Kybybolite sub samples estimated using Manutec® Soil pH Test Kit; $pH_w$ was converted $pH_{Ca}$ using the relationship: $pH_{Ca} = 1.012pH_W - 0.768$ (Conyers and Davey 1988).

The Junee sites were from the lower (J1) and mid slope (J2) of the same paddock. The soil tests from J1 indicate slight acidity ($pH_{Ca}$ > 5.0) from 0-7.5 cm, tending toward moderate acidity ($pH_{Ca}$ 4.6) from 7.5-15.0 cm. Although plants from J1 were well nodulated and roots appeared healthy, root growth remained concentrated in the top 10 cm at early bud development (GS 201), 4 months post emergence. A combination of moderately acidic layers at 7.5-15 cm ($pH_{Ca}$ 4.6) and intermittent waterlogging from July to late September may account for the shallow rooting depth.

The J2 soil tests indicated moderate acidity in the surface 2.5 cm ($pH_{Ca}$ 4.9), tending to severe acidity from 5.0-15.0 cm, with $pH_{Ca}$ ranging from 4.6 at 2.5-5 cm, to 4.1 between 7.5-10 cm. In contrast with J1 plants, J2 plants were yellow and stunted and appeared acutely nitrogen deficient 3 months post emergence. Root growth was restricted to the surface layers (0-4 cm), root hair density was considerably less than on J2 plants, and plants were not as well nodulated. Most plants collected from the J2 site showed symptoms of root disease, in contrast with the relatively healthy J1 plants. It is likely the disease infection was a secondary, physiological response to the more 'hostile' soil conditions (i.e. acidity, waterlogging) at J2. The lower incidence of infection observed in plants from J1 suggests that the higher pH in the root zone may have improved the 'health' of those plants and made them less susceptible to damage and infection. The plants were not screened for specific root diseases, but symptoms indicate the presence of several root disease pathogens, such as Pythium, Rhizoctonia, Fusarium, Phytophthora (K Lindbeck pers. comm.).

The Junee paddock has a history of lucerne pasture, canola and wheat production. In 2013 the traditional approach of bulking multiple 0-10 cm samples from the entire paddock indicated $pH_{Ca}$ 5.4 and failed to detect large horizontal and vertical variability in pH. A blanket lime rate of 1.3 t/ha, applied in 2011 was not incorporated and was insufficient to ameliorate the severely acidic layers at 2.5-10 cm at the J2 site.

The intense pH stratification identified by testing 2.5 cm layers to 10 cm at Holbrook and Junee demonstrated that lime incorporation was ineffective under the minimum soil disturbance sowing systems used. The lime was concentrated in the surface layers and was ineffective in neutralising acidity below about 5 cm depth. Failure to mechanically incorporate lime limits its reactivity and depth of amelioration, and therefore the potential crop response. This is an opportunity cost to growers.

## Conclusion

Effective nodulation is essential to optimise the early growth, vigour and production potential of pulses sown into nitrogen depleted soils. When detailed soil pH data were related to root growth and nodulation of faba bean crops, it was concluded that the presence of severely acidic layers ($pH_{Ca}$ <4.5) was likely to be a major factor responsible for inconsistent 'performance' of this crop on slightly ($pH_{Ca}$ >5.0) and moderately acidic soils ($pH_{Ca}$ 4.5 -5.0), at 0-10 cm. Optimal nodulation requires pH conditions favourable to both the rhizobia and host plant. At the sites recording poor nodulation (Holbrook, Kybybolite and Junee 2), the seed and rhizobia encountered a severely acidic layer ($pH_{Ca}$ 4.4, 4.5 and 4.2 respectively) at the approximate seeding depth of 5.0-7.5 cm. The negative impact of moderately to severely acidic layers on root hair density was apparent in all monitored crops and the pH levels recorded are below those reportedly favourable for rhizobial infection. Our findings highlight the need to review soil testing procedures and acid soil management practices to improve the performance of acid-sensitive legumes on the acidic soils of SE Australia. The traditional 0-10 cm soil sampling procedure is not detecting pH stratification. Finer sampling at 5 cm intervals to a depth of at least 20 cm is needed to verify the presence and depth of acidic layers. Furthermore, the severe acidity at 5-15 cm reported here is likely to reduce the productivity of more acid-tolerant species, including barley, canola, many wheat varieties and pasture species, such as lucerne, clovers and phalaris.

The intense pH stratification identified by testing finer layers demonstrated that lime was concentrated in the surface 0-5 cm layers under the minimum tillage systems used by the majority of participating growers. Further investigation is needed to: (i) assess the effectiveness of current on-farm approaches to acid soil management, and (ii) quantify the impact of pH stratification on more acid-tolerant species and cultivars including canola, cereals and pastures (e.g. lucerne, phalaris and clovers). Our experiences indicate that there is an urgent need to review, validate and update acid soil management recommendations and extension tools in order to improve the efficiency of lime inputs in neutralising acidity in the topsoil and preventing further subsoil acidification.

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