It’s not all about lime – management strategies to improve nodulation and N_2 fixation on acidic soils

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Keywords

- pulse, stress, rhizobia, nodule function

Take home messages

- Maximum nitrogen fixation (N_2 fixation) will only occur if conditions favour rapid development of functional nodules and vigorous plant growth.
- Soil pH was the primary factor limiting the yield and nitrogen (N) contribution of acid-sensitive pulse crops in 83% of commercial paddocks surveyed in central and southern New South Wales (NSW), Victoria (Vic) and South Australia (SA).
- Once low soil pH is ameliorated, fine-tuning of agronomic management that accounts for sowing time, soil type, herbicide usage, nutrition and disease pressure will maximise the opportunity for legumes to fix N.
- Forward planning, effective nodulation and early plant vigour set the production and N_2 fixation potential of pulse crops.

Background

This paper collates observations and data from investigations from 2015 to 2019 by the authors, primarily of commercial pulse crops grown on acidic soils in south eastern Australia. It includes findings from a survey of commercial pulse crops in southern NSW, southern Victoria and eastern SA undertaken as part of a joint GRDC and NSW Department of Primary Industries project The survey aimed to identify factors limiting N_2 fixation and productivity of pulse crops grown on acidic soils in the high rainfall zone (HRZ) of south eastern Australia.

Subsurface acidity was identified as the most significant factor limiting nodulation and production potential of pulse crops, across all rainfall zones. As a result, there has been considerable effort to improve management of acidic soils, and in the selection of rhizobia with the ability to improve nodulation in low pH soils. However, there is a risk that the focus on improving nodulation outweighs the attention needed to improve agronomic management of pulses sown into acidic soils that dominate the cropping zone of central and southern NSW. Eighty-three per cent of our 55 surveyed sites had pH_{Ca}<5.2 in the 0-20cm layers.

The recent success in isolating acid-tolerant rhizobia strains suitable for faba bean and lentil (Ballard et al. 2018) demonstrates tolerance to low pH in rhizobia populations. However, this is not matched by the tolerance of the host species. An effective (N2 fixing) symbiotic relationship between legumes and rhizobia depends on both partners functioning effectively. Most pulse species favour deep, well-drained neutral to alkaline soils (pH_{Ca} 6.0-8.0). Only 17% of the commercial paddocks we surveyed had soil characteristics approaching the optima required by acid-sensitive pulses to achieve their water-limiting yield potential (Burns and Norton 2018a).

Peoples et al. (2001) proposed a 'rule-of-thumb' figure of 20-25kg of shoot N per tonne of above-ground (shoot) dry matter (DM) to estimate the amount of N fixed by legumes. More recently Peoples et al. (2015) reported large variation in the amounts of N fixed by pulses, ranging from <10kg N/ha to > 150kg N/ha, and suggested an average figure for pulses of 19kg of N for every tonne of above-ground DM. They attributed the low quantities of N_2 fixed rate to management and environmental factors, including lack of inoculation at sowing, poor growing conditions due to drought or high concentrations of soil nitrate.

Our investigations highlight considerable opportunities to improve the production potential of pulse crops through attention to fundamental pulse agronomy aimed at avoiding and/or minimising the impact of adverse environmental conditions or poor management decisions. Sub-optimal conditions limit the plant's ability to tolerate and recover from stress 'hits', including cold temperatures, nutrient deficiency, waterlogging, disease infection and/or herbicide injury. The compounding effect of low soil pH on the tolerance of acid-sensitive pulses to these various stress factors they encounter is under-estimated. From our experiences we suggest that this is probably because the stress symptoms are either sub-clinical or attributed to another constraint.

In this paper we outline the steps and processes involved in the establishment of an effective (N2 fixing) legume symbiosis; highlight practices we encountered that compromise the processes and discuss opportunities to improve management practices. Our objective is to optimise effective nodulation and vigorous early plant growth, so as to maximise the production and N2 fixing potential of pulse crops sown into acidic soils of central and southern NSW.

Method

The information presented is based on soil data and assessment of the nodulation and early growth of pulse crops in the 2015 to 2017 growing seasons in NSW, Victoria and SA (Norton et al. 2019), which has been supplemented by additional measurements and observations taken from 2018 and 2019 commercial pulse crops and NSW DPI field experiments.

Results and discussion

Effective legume symbiosis

The negative effect of low pH on rhizobia survival is well publicised. However, the direct influence of low soil pH on the development of pulse roots and nodule formation, and its indirect influence on host plant vigour, DM accumulation and N_2 fixation potential is often overlooked. Maximum N_2 fixation will only occur if conditions favour rapid development of functional nodules and vigorous plant growth. The plant is the source of carbon, i.e. energy for the rhizobia, and the rhizobia is the source of N for the plant; and therefore, failure of either party to function optimally will reduce the benefits of the relationship.

Figure 1 highlights the complexity and interdependence of the processes involved in legume root nodule symbiosis and the development of functional, N_2 fixing nodules. Root infection, nodule formation and N_2 fixation are closely linked to growth and function of both the plant and the rhizobia. Soil condition (temperature, moisture and soil pH) influences each component.

Nodule development follows a sequence of steps involving chemical signalling between the host plant and the rhizobia, accumulation of large numbers of rhizobia at the growing tip of the host root, the production of chemicals by the rhizobia (Nod factors) and the growth and infection of root hairs. All these processes are adversely affected by low pH (Munns 1986).

Inoculation is recommended for acid sensitive pulse crops sown in acidic soils. Good inoculation practices ensure that compatible rhizobia are present in enough numbers, close to the emerging seedling roots. Under ideal soil conditions the rhizobia multiply and colonise the root rhizosphere. Exudates (flavonoids) from the developing root, released in greatest amounts near root tips, act as 'chemo-attractants' and concentrate compatible, host-specific rhizobia adjacent to the growing root tip, near emerging root hairs. The flavonoids activate nodulation genes in the rhizobia, which trigger the rhizobia to attach and infect root hairs and form nodules (Richardson et al. 1988, Abdel-Lateif et al. 2012). (Note: Rhizobia infect most legumes via root hairs, but in some species, including lupin, the site of infection is between cells near emerging lateral roots.)

Once nodules have formed, the amount of N fixed is driven by plant growth rate and their demand for

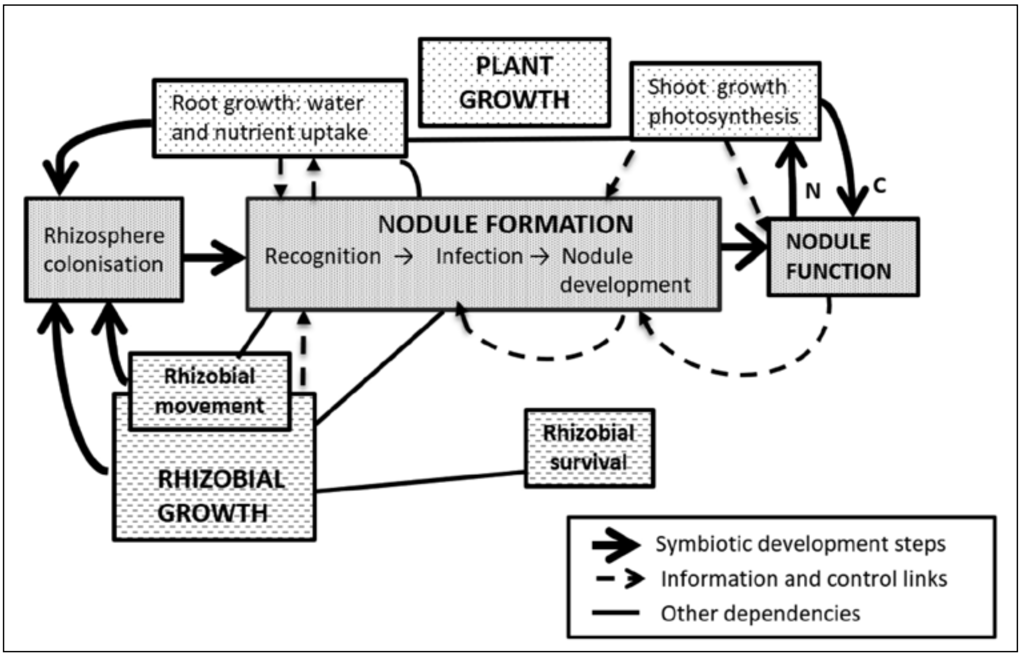

Figure 1. Establishment of effective legume symbiosis is the culmination of an interrelated sequence of events that result in the formation of functional, N_2 fixing nodules. (Source: adapted from Munns (1986)).

N. Therefore, any factor that limits plant growth will limit N_2 fixation potential. Munns (1986) concluded that 'no matter how effectively nodulation has established, nodule function depends on the plant's photosynthesis and growth. Over the long term, plant size (and therefore demand for N) drives nodule mass and number; that plant growth rate dominates total nodule activity (fixation rate)'; and, as observed in our study, 'growth of acid-sensitive pulses is more susceptible to low pH than other phases of the symbiosis, such as rhizobial infection and nodule formation' (Munns 1986).

Managing the impact of stress factors on effective nodulation

Plant vigour and biomass accumulation drives the amount of N fixed by the rhizobia in the mutually beneficial legume symbiotic partnership. Therefore, maximising the N_2 fixation potential of pulses growing in acidic soils depends on minimising or avoiding stresses that compromise plant growth, the growth and function of the rhizobial population, or that disrupt the nodulation process.

Plant responses to the various stresses are often similar, so it can be difficult to pinpoint the direct cause of key failures in the symbiotic relationship, i.e. poor crop growth or nodule formation. For example, physiological yellowing due to herbicide phytotoxicity is a response to disruption of photosynthetic processes but can be confused with N deficiency caused by poor nodulation. It is often too late to take remedial action when symptoms appear, so it is important to be aware of potential issues and actively avoid them.

Stress factors identified as being likely to disrupt the development of effective nodules in pulse crops growing in acidic soils are presented in Table 1. Management options that can minimise the impact of sub-optimal soil pH that dominates southern and central NSW soils are discussed in more detail including; amelioration of soil acidity, improved herbicide management, effective inoculation and sowing time.

Amelioration of soil acidity

The results from the field surveys implicate low pH as the primary factor limiting the potential yield and N contribution of acid-sensitive pulse crops growing in soils with moderately (pH_{Ca} 4.5-5.0) and severely (pH_{Ca} <4.5) acidic layers within 0-20 cm of the soil profile. This is supported by soil and crop data collected from a 2019 field pea crop sown on a historic lime experimental site at Charles Sturt University (CSU), Wagga Wagga.

Figure 2 shows the residual benefit of lime applied in 1989 (at an estimated rate of 2.5 t/ha) that resulted in a significant (P<0.05) increase in soil pH and decrease in exchangeable aluminium per cent (Exch. Al%) in the 0-15cm surface layers, compared with the unlimed treatments. The pH_{Ca} in the 0-5, 5-10, 10-15 and 15-20cm layers for the limed plots was in the moderately acidic range (5.2, 4.5, 4.6 and 4.9, respectively) compared with more severely acidic layers in the unlimed plots (pH_{Ca} of 4.4, 4.1, 4.2 and 4.6, respectively). Under the exceptionally dry conditions of 2019 (decile 1) the 1989 limed plots produced more than twice the DM than the unlimed plots, from an early September cut, at peak biomass: 2.14t DM/ha compared with 1.01t DM/ha; and almost four times more grain yield than the unlimed plots: 0.42t/ha from the lime plots compared with 0.11t/ha from the unlimed plots (Figure 3).

Clearly, low pH in the surface 0-20cm soil layers places a yield ceiling on acid-sensitive pulses, reinforcing the necessity for a long-term approach to acid soil amelioration if pulses are to become a viable option in cropping systems of southern and central NSW. It is important to note that field pea is considered the most acid tolerant of the pulses; and although the grain legumes, albus and narrow-leaf lupins, are generally considered acid tolerant, productivity of these species would also have been compromised at the 17% of the survey's severely acidic sites (Burns et al. 2018a) with profiles similar to the unlimed plots at the CSU site (M Richards pers.comm.).

Herbicide management

Increased herbicide options and improved opportunity to manage herbicide resistance were ranked as primary reasons given by growers and advisors for including pulses in cropping programs. However, there is limited data on the effect on nodulation and N_2 fixation of herbicides used in pulse crops growing in soils with sub-optimal pH. Our field observations suggest that a focus on weed management and the use of high rates of herbicide could be compromising pulse crops.

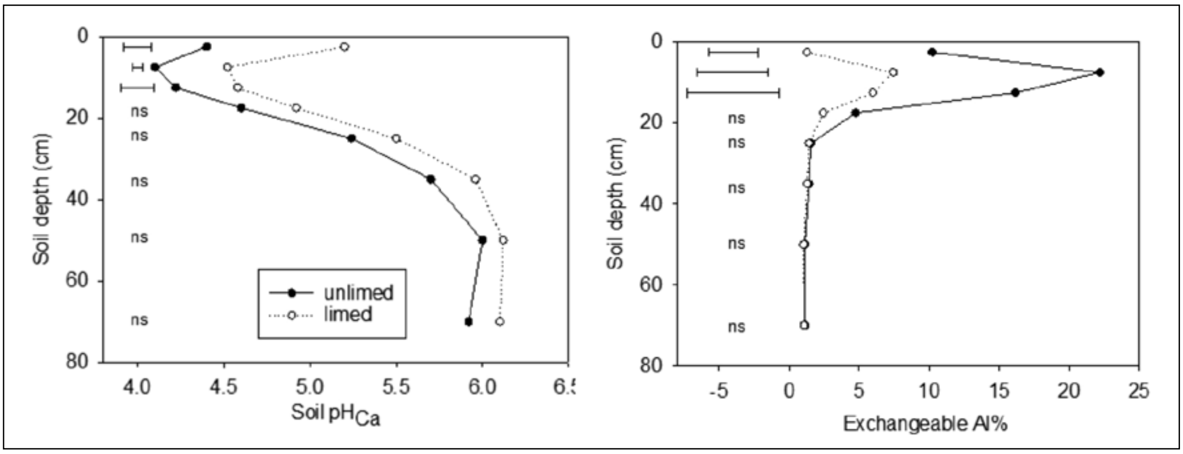

Figure 2. Soil pH and exchangeable Al% measured in 2019 showing the residual benefit of lime applied in 1989 at Wagga Wagga, at a rate of 2.5t/ha. Horizontal bars in represent Lsd for each sampled depth layer, ns = no signifcant difference (P<0.05).

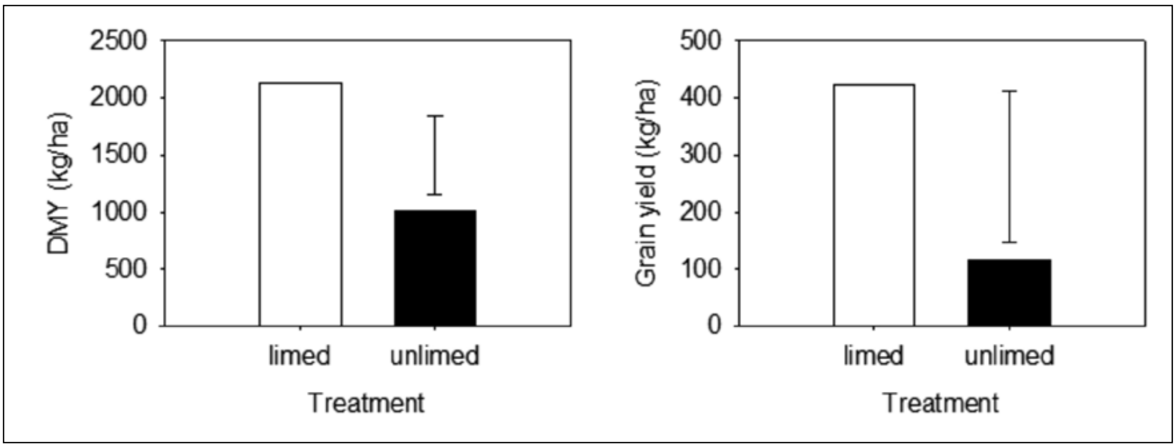

Figure 3. The residual benefits of lime applied in 1989 resulted in a significant increase in dry matter yield (DMY) and grain yield from field peas sown in 2019 compared with unlimed plots. (P<0.05, Lsd=702 for DMY; Lsd = 264 for grain yield)

Table 1. Stress factors that may reduce nodulation in legumes by disrupting key phases in the pulse-rhizobia symbiosis shown in Figure 1: rhizobial growth, plant growth, and nodule formation and function. Low pH directly or indirectly influences every part of the symbiosis.

Phase	Stress factor	Impact
Rhizobial growth	Poor inoculant storage, handling or inoculant application method – follow inoculant label guidelines	Lower rhizobia population, delayed nodulation
	Pesticides, fungicides, trace elements and fertiliser compounds in direct contact with rhizobia: - check pesticide and fungicide label recommendations for compatibility with rhizobia before treating seed - trace elements such as zinc and copper are toxic to rhizobia - Do not mix sodium molybdate with inoculant. Only use molybdenum trioxide or ammonium molybdate. Avoid direct contact of rhizobia or inoculated seed with fertiliser.	Prolonged exposure of rhizobia to incompatible chemicals and pesticides can result in nodulation failure
	Low pH	Reduced rhizobia survival, which delays and reduces nodulation.
	Cold temperatures – e.g. delayed sowing	Slows rhizobia colonisation and nodule activity (N2 fixation rate)
	Dry conditions at sowing	Rhizobia numbers applied to seed will decline in extended dry conditions.
Plant growth	Low pH	Reduces signalling between host plant and rhizobia.
		Restricted root growth and limited opportunity for rhizobia infection.
		Reduced plant vigour limits N_2 fixation potential.
	Cold temperatures	Delayed rate of nodule formation
		Plant growth and N_2 fixation slows as temperature declines
	Herbicide injury caused by: - carryover of herbicide residue from previous crop(s); - physiological stress and crop yellowing caused by in-crop herbicides.	Root damage limits rhizobial infection sites.
		Phytotoxic effect reduces plant vigour and photosynthetic capacity
Nodule formation and function	Low pH	Disruption of several steps in the nodule formation pathway reduces nodule number and nodule mass.
	Herbicide injury	Root damage limits the number of rhizobia infection sites.
		Crop yellowing reduces photosynthesis, nodule activity and N_2 fixation.
	High soil nitrogen	Moderate levels of soil N reduce N_2 fixation. High levels (>50 kg N/ha in the root zone) will reduce nodulation.
	Cold temperatures	Rhizobial activity and N_2 fixation declines as soil temperature drops. It is negligible below 10°C
	Host plant has limited access to moisture and nutrients	Reduced plant vigour and growth rate will reduce N_2 fixation

Note: Molybdenum (Mo) is a micronutrient that is essential for production of enzymes needed for N fixation. Mo availability declines as pH decreases and is most likely to be deficient in soils of $pH_{Ca} < 5.0$.

These concerns reflect the findings of Drew et al. (2007) who investigated the impact of herbicides in sub-optimal growing conditions, i.e. low rainfall, on nodulation, N_2 fixation and grain yield in field pea in the low rainfall zone of SA. The experiment was conducted on calcareous loam soils (pH_{Ca} 8.1).

They reported herbicide damage from registered herbicides applied at registered rates, which they concluded was a consequence of a slow growth rate and reduced ability of the drought stressed plant to metabolise the herbicide rapidly into an inactive form. They found that reduced nodulation following herbicide treatment 'was most likely an indirect effect of the herbicide on the plant'. This agrees with observations of root pruning and poor nodulation in lentils growing in light-textured, acidic soils. This crop had received rates of herbicide suitable for heavy clay soils. Close inspection of affected plants indicated that root pruning as a result of excessive herbicide rates and therefore absence of root hairs and potential rhizobia infection sites was the most likely reason for the poor nodulation.

Drew et al. (2007) noted that in-crop yellowing can be indicative of stress to the legume symbiosis, with reduced photosynthetic capacity of plants exposed to herbicide damage resulting in reduced carbon supply to nodules and therefore reduced nodule activity and slow plant growth rate. They also suggested that because 'reduced nodulation or N_2 fixation was sometimes evident in the absence of yield reductions, (grain) yield should not be the sole measure of herbicide tolerance in peas.'

These findings raise concerns about the acceptance of a crop yellowing response to herbicides commonly observed in pulse crops in this region and the potential impact of some herbicide programs on N_2 fixation. As suggested by Drew et al. (2007) particular care should be taken in the selection of herbicides used in legumes grown as break crops for the purpose of their N benefit. The risk of herbicide injury is likely to be increased for pulses growing in acidic soils, where plant growth rates and legume symbiosis may already be compromised. Strict adherence to herbicide application guidelines and rates is essential, particularly regarding plant-back intervals and matching rates to soil type.

Rapid breakdown of sulfonylurea (SU) herbicides in acidic soils means that the risk of carry-over of residues from one season to the next has not been of concern on the acidic soils of NSW. However, because stratified soil pH with an elevated pH in the surface 0-5 cm layers was a common feature of the commercial paddocks we surveyed (Burns et al. 2018b), growers should be aware of their soil pH profiles and the risk of SU residues when sowing SU sensitive legumes: SU sensitive legumes should not be sown in paddocks on which SU herbicides have been used in the last 12 – 24 months.

At greatest risk of carry-over of SU residue are those soils with a recent lime application and a history of minimum tillage (Burns et al. 2018b). Elevated soil pH profiles, e.g. pH_{Ca} 6.0 in the surface 0-5cm layer, falling to just 4.5 at 5-10 cm layer, were common in our survey, but not detected from samples collected at the traditional sampling depth of 0-10cm. In this case the 0-10cm pH_{Ca} would be 5.3 and apparently safe for a replanting interval of 12 month. According to triasulfuron guidelines 12 months is the replanting interval when pH_W (1:5 water method) <6.5, which is approximately pH_{Ca}<5.7. However, when pH_W>6.5 (i.e. pH_{Ca}>5.7), the replanting interval can be up to 22 months.

The inoculation procedure

Our field surveys did not identify any link between poor nodulation and the form of inoculant used by growers surveyed. The most significant cases of nodulation failure caused by shortcomings in the storage and handling of the inoculant and when zinc was mixed with the rhizobia prior to inoculation.

Many pesticides, fungicides, trace elements and fertilisers are toxic to rhizobia. Check pesticide and product labels for compatibility with rhizobia before mixing.

Sowing time

Timely sowing of pulse species, early in the recommended sowing window, is particularly important in challenging acid soil environments. Experience with other acid-sensitive plant species e.g. lucerne, barley and canola, suggests that early sowing when temperatures favour rapid growth, allows the sensitive seedlings to establish, accumulate leaf and develop a robust plant that is tolerant to hostile environmental conditions such as cold, waterlogging, acidity and other stresses including herbicide injury and disease pressure.

Pulses also benefit from early establishment of an effective legume symbiosis. Growth and development of the rhizobial population, N_2 fixation and plant growth all decline as temperatures approach and fall below 10°C, when activity is negligible. Delayed sowing will limit the period of high N_2 fixation rates to the end of the growing season, when temperature and plant growth rates increase.

Conclusions

We have identified deficiencies in the management of pulse crops sown in south-eastern Australia that are limiting their productivity, N contribution and expansion into moderately acidic soils. We acknowledge that much of this is based on anecdotal evidence, mainly because there is limited research data that specifically addresses the agronomic challenges facing pulse production in acidic soils under contemporary farming systems. However, we have highlighted opportunities, through increased attention to agronomic management, in conjunction with a long-term acid soil management program, to optimise the grain yield and N contribution of pulse crops in southern and central NSW.

Acid soil amelioration efforts will increase pH. However, under current practices, the soil pH within the surface layer at depths of 0-20 cm of most soils in the southern and central NSW is likely to remain below the optimal pH range of pulse species (i.e. pH_{Ca} 6.0-8.0).

Forward planning and management are critical for maximising the productive potential and N contribution of pulses grown in acidic soils. Fundamental to this is paddock selection based on an understanding of soil types, specifically pH of the soil profile, and efforts to minimise any stresses that could reduce early plant vigour and rhizobial activity. The checklist below summarises key soil, and agronomic management guidelines to consider when sowing pulses into low pH soils:

1. Start planning at least two years before sowing acid-sensitive pulses.
2. Check for the presence of acidic layers at 0-20 cm; a garden soil test kit provides a quick and cheap indicator of acidic layers which can then be quantified with soil testing. Have samples analysed by an accredited laboratory, ideally collected at 5 cm intervals to a depth of 20 cm.
3. Avoid sowing acid-sensitive pulses into soils with severely acidic pH to depth; use soil test data to guide acidic soil management strategies; implement liming programs at least 12-24 months before sowing sensitive species to allow time for lime to react and increase pH. There are long-term benefits to pulse crops from lime application.
4. Avoid sowing acid-sensitive pulses in soils with a shallow topsoil and impermeable subsoil that is prone to waterlogging or impedes root development.
5. Avoid paddocks with heavy infestations of broadleaf weeds or herbicide resistant grass weeds that cannot be effectively controlled by a combination of pre-sowing control and in-crop herbicides.
6. Check herbicide use in the previous 12-24 months and ensure maximum plant-back periods are satisfied, with particular attention to Group B, chlopyralid and triazine herbicides:
   - Chlopyralid persists in residue of treated crops and may severely damage legume species. Check replanting intervals on herbicide labels.
   - Avoid sowing sulfonylurea (SU) sensitive legumes in paddocks on which SU herbicides have been used in the last 12 – 24 months.
   - Check herbicide rates: high rates on the light soils typical of the region may cause phytotoxicity, including root pruning in sensitive crops, which will reduce nodulation and N_2 fixation.
7. Minimise disease risk in pulse crops by following cropping interval and crop separation guidelines.
8. Check seed quality. Time of harvest, moisture at harvest, header set up, seed size and post-harvest storage conditions can all impact on seed quality, seedling vigour, crop yield and N contribution.
9. Sow on time, early in the recommended sowing window, to allow plants and nodules to establish before cold temperatures slow growth and rhizobial activity.

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