Invited paper: Lupin genetic improvement for targeted environments and markets

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Abstract

The narrow-leafed lupin (*Lupinus angustifolius* L.) is a wild native species of the Mediterranean region that has become the dominant grain legume crop in Western Australia. Domesticating the plant for mechanised agriculture is a classic plant breeding story involving conventional breeding techniques. Further integration into the farming system, expansion of the crop and widespread utilisation of the grain has involved cooperation between breeders, agronomists, grain chemists and marketers.

In the 1980's lupinosis disease in livestock, caused by mycotoxins produced on lupin stubble by the phomopsis fungus (*Diaporthe toxica* Williamson) limited the adoption of lupins into mixed cropping and grazing systems. Breeding for resistance to phomopsis was a priority and the successful development of the first resistant varieties Gungurru and Merrit allowed lupins to be widely grown. Differences in biotic stresses between high and low rainfall regions became apparent as lupins were grown in tight rotations. Lupin anthracnose (*Colletotricum lupini* (Bondar) Nirenberg, Feiler & Hagedorn)is an important yield affecting disease in the high rainfall zone and breeding produced the first resistant varieties Wonga and Tanjil. In contrast, anthracnose resistance is not as critical in the low rainfall zone where relatively simple agronomic practices give adequate control. Cucumber Mosaic Virus (CMV) infection is most problematic in the high rainfall zone. Screening techniques were developed that have identified lines that have low transmission rates, which in conjunction with agronomic practices, minimise losses due to CMV. In the low rainfall zone brown spot (*Pleiochaeta setosa* (Kirchn.) Hughes) and aphid colonisation (*Aphis craccivora* Koch., *Acyrthosiphum kondoi* Shinji) are major yield affecting biotic stresses. Screening techniques for brown spot have been developed and moderate resistance has been identified. Resistance to aphid colonisation is related to alkaloid content and there are varieties with excellent resistance to these aphid species. Regional biotic stress requirements are matched to regional requirements for harvestability, herbicide tolerance and yield.

Low alkaloid content was the essential quality parameter required to gain acceptance into feed and food markets. Although all varieties carrying the *icundus* gene are ‘sweet’, significant genotypic and environmental variation in seed alkaloid levels still exist. A continuous monitoring of alkaloid levels across years is required to identify lines that are consistently below the required benchmark. Genetic improvement to increase protein and reduce hull proportion has been initiated with a view to establishing new markets and processing opportunities.

Key Words

Narrow-leafed lupins, phomopsis, anthracnose, brown spot, Cucumber Mosaic Virus, aphids, alkaloids, grain quality

Introduction

Narrow-leafed lupin (*Lupinus angustifolius* L.), which belongs to a group referred to as the ‘Old World’ lupins, evolved in the Mediterranean basin. Of this group *L. luteus* L. and *L. albus* L. were first domesticated as a grain crop in Germany in the early 20th Century, having been used extensively before that as a green manure or forage crop.

L. cosentinii Guss. was introduced to Western Australia (WA) in the mid to late 19th century and was utilised as a pasture species in the Geraldton region on sandplain soils. John Gladstones began evaluation of all these species in the 1950s before focussing on the development of the narrow-leafed lupin (NLL). At this time another NLL breeding effort was evolving in the United States Department of Agriculture based at Tifton, Georgia which provided a source of collaboration. More recently the lupin breeding in Germany, Poland, Denmark, Belarus and Russia has swung in favour of NLL at the expense of *L. luteus* and *L. albus*, particularly since the arrival of anthracnose disease in that part of the world.

In 1959 the only narrow-leafed lupin variety available to Australian farmers was the forage variety, Borre. While this variety possessed sweetness (low alkaloid content) and soft (permeable) seeds it lacked non-shattering pods and was too late maturing for most areas. In the 1960s Gladstones (1967) reported the discovery of two naturally occurring mutant genes for reduced pod shattering. These were independently inherited recessive genes: *lentus*, which reduced the endocarp thickness of the pod walls, and *tardus* which fused the sclerenchyma strip of the dorsal and ventral pod seams. When these two genes were combined they produced a non-shattering type that could withstand desiccation. At the same time a mutant gene (*Ku*) for early flowering was discovered which removed the requirement for vernalisation (Gladstones and Hill 1969). This single dominant gene allows NLL to flower 2-3 weeks earlier in colder districts of south-western Australia and by 3-5 weeks in warmer districts.

The discovery of the non-shattering genes and the early maturity gene enabled the domestication of NLL to be completed. Gladstones also added two genetic markers (white seeds and white flowers) to the breeding program so that fully domesticated varieties could be easily differentiated from naturalised semi domesticated varieties or wild types (grey seeds and blue flowers). Contamination at receival points could then be identified and ensured Australian varieties could be marketed with confidence as having low alkaloids and thus suitable for a wide range of stockfeed uses.

The early varieties, Uniwhite (1967), Uniharvest (1971) were late maturing varieties while Unicrop (1973) was the first of the early maturing varieties incorporating the *Ku* gene.

The 1970's was a pioneering decade for lupin production with most of the research effort going into the production techniques for growing a successful lupin crop. Production rose by 1975 and peaked at approximately 90,000 tonnes before a substantial contraction back to 20,000 tonnes due to dry seasons, disease and weed management. There remained a strong desire to make lupins succeed as a rotation crop on the sandplain soils of the northern agricultural region of WA as wheat monoculture was afflicted with high levels of foliar disease and increasing burdens of brome grass. Also, grazing of pastures on these fragile soils often led to serious wind erosion.

It was during the 1980s that lupins went through a period of rapid growth as a result of matching the improved varieties Illyarrie and Yandee with an improved agronomic package based on early sowing, more effective use of the herbicide simazine and direct drilling technology into cereal stubbles. There were peaks and troughs in grower confidence as challenges emerged from diseases such as phomopsis, root rots, brown spot, CMV and anthracnose.

Challenges of the environment and farming systems

Biotic stress at the whole farm level - lupinosis

In the 1970's and 80's lupinosis disease in livestock, most notably sheep grazing on lupin stubble, became prevalent. Stock losses due to this disease caused alarm amongst farmers and affected the adoption of lupins into mixed cropping and grazing systems.

Gardiner *et al* (1970) were the first to demonstrate that lupinosis was caused by a myctoxin produced by the fungus *Phomopsis leptostromiformis* (Kuhn) Bubak (now called *Diaporthe toxica* Williamson). Sheep grazing stubble over summer were most vulnerable to this disease when summer rainfall promoted fungal growth and the production of toxin. The risk of lupinosis was significant enough to discourage many farmers from growing lupins. Obviously for lupins to become a significant grain legume in the mixed farming system a solution to this problem was needed.

The early varieties of lupins were described as susceptible to the disease (Wood *et al* 1975). Careful management was needed to reduce the risk of lupinosis for sheep grazing these stubbles. Grazing practices were changed (Gardiner 1975, Allen 1982), fungicides were sprayed throughout the season and on stubble (Wood *et al* 1975), and mixtures of susceptible and resistant varieties were sown. The *L. albus* variety Ultra was noted as being resistant and did not produce the fungus on the stubble (Wood and Allen 1980). Breeding for resistance to the fungus was seen as the more effective solution to the problem and became a priority.

On evaluating the wild and introduced germplasm it was found that resistance to phomopsis stem blight was present in some accessions from Mediterranean countries. A proportion of accessions from Morocco, Italy, Spain and Portugal all had a high level of resistance. The resistance in the wild accessions was found to be inheritable and resistant breeding lines were produced and tested across environments. The best lines were also tested for their lupinosis toxicity to sheep across a number of environments and years. There was markedly reduced toxicity to sheep of stubble from resistant compared to susceptible lines (Allen *et al* 1985). In 1988 the first phomopsis resistant variety, Gungurru, was released. The variety was heralded as a new era in the lupin industry that saw the wider adoption of lupins on farms where livestock enterprises were more important.

The benefits of phomopsis resistant lupins, like Gungurru, was seen through a reduction in sheep deaths, a longer safe grazing period on lupin stubble, a decrease in the need for supplementary feed over summer and improvements in sheep body weight and possibly wool growth (Warren *et al* 1989). They also estimated that net farm income from growing the new variety Gungurru compared to Danja would increase by $3,000 because of the extended period of grazing provided to Gungurru. It is also evident that phomopsis resistance can reduce lupin production loses in situations where pre-harvest stem or pod infection is severe and certainly has reduced the incidence of phomopsis discoloured seed in harvested grain. There is a receival standard maximum of 3% discoloured seed for stockfeed markets and a zero tolerance for food markets.

Since the release of Gungurru, all lupin varieties have resistance to phomopsis and it remains a key breeding objective. Heritability across sites is high (Cowling *et al* 1987) and this allows simple field selection or selection using a seedling screening technique (Williamson and Sivasithamparam 1991) for advanced lines. There are two well characterised genes for resistance to phomopsis stem blight (Shankar *et al* 2002) and evidence that some combinations of these and other genes can lead to transgressive segregation. Whilst lupins remain a key grain legume in a mixed farm systems the question of what level of resistance is necessary or acceptable continues to be debated and changes depending upon the value of wool and meat.

Biotic stress in the high rainfall region

(a) Anthracnose

Lupin anthracnose caused by the fungus *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn was first detected in Australian crops in 1996 (Sweetingham *et al* 1998). It rapidly became a very serious disease in the high rainfall northern agricultural zone of Western Australia, particularly in districts where WA blue lupins (*L. cosentinii*) provided a source of ongoing infection. Breeding for resistance to anthracnose in NLL began in the south-east USA in the 1950s and resulted in the release of varieties such as Rancher, which carried a single dominant resistance gene (Wells and Forbes 1967). Gladstones (1982) reported the incorporation of the resistance gene from Rancher into the Australian variety Illyarrie. However, Illyarrie is only intermediate in resistance (score 5) when challenged by the anthracnose fungus that was found in Western Australia in 1996. The level of resistance (score 7) in varieties Wonga and Tanjil which were released in WA the late 1990s is much greater and comes from a different source. It has now been shown (Yang personnel communication) that there are at least two dominant, independent and non-additive genes for anthracnose resistance in the breeding program. The two genes, *Anr1* and *Anr2* are found in Tanjil and Mandelup, respectively.

Irrigated disease nurseries and more recently molecular markers (Yang *et al* 2004) are used as screening tools by the breeding program to maintain an appropriate level of anthracnose resistance in breeding lines and future varieties (Tivoli *et al* 2006).

Detailed yield loss experiments (Thomas and Adcock, 2002; Thomas and Sweetingham 2004) demonstrated that anthracnose was much less acute in lower rainfall environments and that a lower level of resistance in NLL was adequate, particularly if clean seed and fungicide seed dressings were used. Varieties such as Belara, which is considered ‘moderately susceptible’ (score 4), has adequate resistance in low rainfall (< 350mm) zones and the ‘moderately resistant’ Kalya (score 6) has adequate resistance in medium rainfall (350-450mm) zones in Western Australia. Only resistant cultivars like Tanjil and Wonga (score 7) are adequate for the high rainfall "hot spot" for anthracnose around Geraldton and Dongara.

(b) Cucumber Mosaic Virus

There are two important virus disease in lupins, bean yellow mosaic virus (BYMV) and cucumber mosaic virus (CMV). Both viruses are transmitted by aphids but only CMV is seed-borne in NLL. Seed borne CMV becomes the major source of infection in a crop and the percentage of seed infection is directly related to the level of potential spread through a crop by aphids. CMV infection is most problematic in the high rainfall zone where it can lead to serious yield losses.

The aphid species involved in the spreading of CMV in lupins are the colonising species, *Myzus persicae* Sulz (green peach aphid), *Acyrthosiphon kondoi* Shinji (bluegreen aphids) and *Aphis craccivora* Koch (cowpea aphid) and the non-colonising species *Rhopalosiphum padi* L. (birdcherry-oat aphid), *R. maidis* Fitch. (corn leaf or maize aphid) and *Lipaphis erysimi* Kaltenbach (turnip aphid) (Jones and Proudlove 1991, Bwye et al 1994, Berlandier et al 1997). The non-colonising aphids were found to contribute more to the spread of CMV than the colonising aphids because they tend to probe several plants before moving (Berlandier et al 1997). Aphid numbers are greatest close to the coast where moderate winter temperatures and higher rainfall suits their life cycle. Summer rainfall or early breaks to the season can result in early aphid build-up and greater spread of CMV in the crop.

Yield losses due to CMV is related to the earliness of initial spread within the crop, which is in turn related to the level of seed infection and the timing of aphid flights. An annual CMV forecasting model (www.agric.wa.gov.au), based on the pattern of summer or pre-cropping rainfall, can be used to show the risk of early aphid arrival. The level of seed infection and aphid infestation of crops can be influenced by agronomic practices and through breeding. Early sowing, high seeding rates and narrow row spacing can encourage the establishment of a dense canopy, which can shade out stunted CMV infected seedlings. The retention of stubble decreases the number of landings of early flights of aphids compared to bare ground (Bwye *et al* 1995). The sowing of seed with no CMV infection (high rainfall or high risk region) or less than 0.5% (low rainfall or low risk region) is recommended to reduce the yield losses (Bwye *et al* 1994, 1995).

Screening techniques for seed transmission of CMV were developed and have identified lines that have low seed transmission rates (Jones and Cowling 1995). Although resistance to seed transmission is likely to be a polygenic character, improvement in resistance is possible through breeding. Field testing has shown that Danja, Tanjil and Wonga are moderately resistant and have low (4%) levels of CMV seed transmission; Gungurru and Illyarrie are moderately susceptible (11%) and Wandoo is susceptible (>20%). All advanced breeding lines are now screened for CMV seed transmission to eliminate any lines that are susceptible and to understand the risks for new varieties.

Farmers in the high rainfall northern areas, where the risk of yield losses from seed-transmitted-CMV is high, are encouraged to grow the most resistant varieties, to use clean seed and to apply the appropriate management that discourages aphid landings and disadvantages weak, CMV infected seedlings.

Biotic stress in the low rainfall region

(c) Brown spot

Brown spot is caused by the rain-splash of soil-borne spores of the fungus *Pleiochaeta. setosa* (Kirchn.) Hughes up onto the cotyledons and leaves. Early seedling infection can severely set back the crop and reduce stand density through plant death. Epidemics that develop later in the season are less damaging but can still reduce yield significantly. In the 1:1 (wheat:lupin) rotations of the 1980s the build up of spores in the soil was significant. The impact of the disease on crop yield is highest in the low rainfall area which is cooler with a short growing season. Here the lupins remain smaller and exposed to rain-splash for longer and there is less opportunity to compensate for early reductions in vigour (Sweetingham *et al* 1998). Brown spot is usually associated with a root disease (Pleiochaeta root rot) caused by the same fungus (Sweetingham, 1984).

The impact of brown spot on lupin production was reduced through research that improved agronomic management and to a minor extent by breeding. Retention of cereal stubble is recommended to reduce rain-splash of the spores from the soil and fungicide treatment of the seed gives the seedlings protection at their most vulnerable stage (Sweetingham *et al*, 1993). Direct drilling NLL accurately at 5cm was shown to minimise Pleiochaeta root rot as this allowed the seed to be placed below the majority of spores and prevent infection of the roots. No sources of strong resistance to either brown spot or Pleiochaeta root rot were found in the breeding program or have been identified in wild germplasm. However, evidence of minor gene resistance was found (Cowling et al, 1997). Through a recurrent breeding approach the variety Myallie was released which shows reduced defoliation due to brown spot in mature plants. However, there is no evidence that the reduced defoliation in Myallie has any protective effect on seedlings, nor a yield advantage under strong brown spot infections later in the year.

A glasshouse based screening technique for Pleiochaeta root rot has been developed and is used routinely on advanced lines in the breeding program. The development of a consistent screening test for brown spot using artificial inoculation of the soil in irrigated nurseries continues to occupy plant pathologists. Emphasis on breeding for resistance to these diseases has been reduced owing to a limitation on resources and a reduction of importance in these diseases associated with a widening of the rotation.

(d) Aphid colonisation

Colonisation by aphid species is a significant problem in the low rainfall zone. *Aphis craccivora* (cowpea aphid) and *Acyrthosiphum kondoi* (bluegreen aphid) most commonly build up on susceptible varieties in the low rainfall areas causing loss of yield.

Berlandier and Sweetingham (2003) studied the yield loss in four NLL varieties due to aphid damage at four sites across Western Australia. They found that aphid damage varied greatly but was significantly influenced by lupin cultivar. Yields for the same treatment combination also varied considerable across geographical locations. Susceptible varieties like Yorrel suffered complete loss of yield at Merredin but only small losses at the coastal sites of Geraldton and Badgingarra, whereas, the resistant variety Kalya did not show any significant loss of yield at any of the sites. They also found that one strategic spray of aphicide controlled aphids and increased yield by as much as 95%. Their conclusion was that farmers needed to be aware of the susceptibility of their chosen variety and that a strategic spray was economical to control aphid colonisation and prevent yield loss.

Resistance to aphid colonisation is related to alkaloid content and there are varieties with excellent resistance to these aphid species. The varieties like Mandelup, Tanjil, Wonga and Kalya have excellent resistance against the two colonising aphids in the low rainfall. All these lupins have alkaloid levels at the higher end of the acceptable range. While breeders have bred resistant varieties it is only more recently that entomologists have investigated the mechanisms. Edwards et al. (2003) found that the resistance mechanism did not influence host selection and acceptance, but had a deleterious effect on the subsequent development of late instars and apterous adults in *A. kondoi* and *A. crassivora*. In other words, resistant varieties reduced the growth and reproduction of aphids, but did not deter them from visiting the plants in the first place. However, they noted that *M. persicae* survival and growth did not differ significantly between resistant and susceptible NLL varieties. *M. persicae* is the least common of the three aphid species to colonise lupins in Western Australia (Berlandier and Sweetingham 2003) which means that it is not a great threat to the resistant varieties in the low rainfall area.

Grain chemistry and marketing

Low alkaloid content is the essential quality parameter required for NLL to gain acceptance into feed and food markets. NLL are sold on the international feed market as Australian Sweet Lupins (ASL) as an indicator of high quality and the guarantee that total alkaloid concentrations < 200mg/kg (0.02%). This standard ensures that lupins are a safe feed for cattle, sheep, pigs, poultry and fish. In the stockfeed industry it has been shown that pigs are sensitive to alkaloids above 0.02% (Godfrey et al 1985). In addition, this level has been accepted in Australia and New Zealand as the maximum level for human consumption (Food Standards Codes, at www.anzfa.gov.au/foodstandards).

Although all varieties carrying the *iucundus* gene are 'sweet', significant genotypic and environmental variation in seed alkaloid levels still exist. Harris (1994) reported that seed alkaloids ranged from 0.002% to 0.090% across 90 receival points throughout Western Australia. In 1988/89 season the alkaloid levels in lupin seed was examined more closely at Wongan Hills. Harris (1994) reported that every delivery was sampled and analysed for alkaloid content and the range was from 0.001% to 0.052%, although he did not record which varieties were delivered. The mean alkaloid for the receival point was 0.009%. On measuring the lupin seed alkaloid levels sampled from 46 truckloads from one individual farmer he again found a range of 0.001% to 0.038%. It is accepted that even though seed alkaloids vary from year to year and location to location that the breeding process for releasing lupin varieties results in varieties that when delivered to market the average alkaloid level of the exported grain is well below the acceptable standard.

It is known that alkaloid content in seed is influenced by a number of factors, including high temperatures during seed development, insect stress during the season and nutritional factors, particularly K and P. Gremigni *et al* (2003) showed that alkaloid levels were high when K was deficient and that when P and K were abundant alkaloid levels were low. Some of the variation noted by Harris (1994) may be explained by variation in soil fertility.

Cowling and Tarr (2004) analysed lupin samples taken from 126 field trials in Western Australia over 11 years at 55 locations and showed that the environment had a greater effect on lupin seed quality than genotype. Genotype, year and location affected the variation in total seed alkaloids. Some varieties (Danja) had greater variation than others (Yorrel and Myallie), while some years were higher than others (range 53-213 mg/kg), and some locations were higher than others (range 47-217 mg/kg). These results highlight the difficulty in breeding for low alkaloids based on single site/year alkaloid data.

Breeding for low alkaloids creates a problem when alkaloid content can vary across sites and years. The cultivar Danja on average has alkaloid levels close to 0.02% and it is accepted as an internal standard in the breeding program. All advanced lines are grown at the same site with Danja with some other controls and the level of alkaloids is compared to the controls. Early generation lines that have seed alkaloid levels greater than Danja or the other controls are usually eliminated from the program. For lines that have progressed through this culling phase and entered the Crop Variety Testing (CVT) alkaloid levels are measured across sites and years. Only lines showing a consistent lower alkaloid level than Danja in these trials are released.

Genetic improvement to increase protein and reduce hull proportion has been initiated with a view to establishing new markets and processing opportunities. Until recently high protein concentration was not a formal breeding objective since most lupin varieties fall within the acceptable range (33-35% dry matter basis). Market analysis has identified protein sensitive markets, such as in the aquaculture industry, which require higher protein levels. Gladstones and Crosbie (1978) showed seed protein content (N x 6.25) of NLL in the genetic resources ranged from just under 32% to almost 43% (dry matter basis), with the majority of lines between 33% and 38%. The impending release of breeding line WALAN2173M in 2006 creates new opportunities for high protein markets, as this variety is 2-4% higher in protein than the current varieties. While breeding for higher seed protein content *per se* will allow NLL to reach new markets, like aquaculture, lupins will still compete against other vegetable protein commodities. Research continues to define unique properties of the lupin proteins so as to distinguish it in the marketplace and to maximise the value and return to the industry. Evidence is emerging for excellent functional emulsifying and foaming properties of lupin proteins in food systems (Holey *et al* 2001).

For aquaculture NLL needs to be dehulled. The hulls make up 25% of the whole seed, compared to other grain legumes such as field pea (*Pisum sativum*) (12%). Clements et al (2004, 2005) have recently identified germplasm with less hull proportion and some of these lines are now being integrated into the breeding program. A NIR technique has been developed for measuring hull proportion and this is being used to assist the selection of thinner hulled lines (Burridge and Sipsas, unpublished).

Other opportunities exist for exploiting the unique components of the lupin seed such as non-starch polysaccharides (dietary fibre), lutein, tocopherols (antioxidants), and breeding awaits evidence of genetic variation and better methods for large scale rapid screening of these components before they can become a commercial breeding objective.

Conclusion

With the basic domestication of the NLL completed about 30 years ago, substantial improvements in yield have continued through selection across a range of high and low rainfall environments. Concurrent with this progress has been an improved understanding of the key abiotic, disease and pest constraints for NLL in these environments by collaboration with agronomists, plant pathologists and growers. There has been little evidence of regional adaptationand watershed varieties such as Gungurru were grown throughout Western Australia and also had an impact in the eastern states. It seems likely that we may soon see varieties released for specific environments based on length of season, temperature and related pest, disease and weed spectrums.

Perhaps the biggest challenges still remain given the scenarios of increasingly complex weed management with the development of herbicide resistant weeds, and static or the declining returns from export protein markets, in part due to competition from the huge rise in global soybean production. The breeding program is already improving herbicide tolerance, through the release of new varieties such as cv Mandelup (improved tolerance to metribuzin) and broadening herbicide tolerance. In addition selection for greater early biomass may be possible in the low rainfall region as a counter to the weed management problems. Improvements in grain quality are achievable within limits, but the extent to which this can benefit the industry will depend on our ability to interpret market signals from sectors where a premium is likely to be paid.

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