



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

The Biology of *Lupinus L.* (lupin or lupine)



Photograph courtesy of Alan Meldrum, Pulse Australia

Version 1: April 2013

This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

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PREAMBLE

This document describes the biology of *Lupinus L.*, with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *Lupinus* species, general descriptions of their morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified (GM) *Lupinus* species that may be released into the Australian environment.

Lupinus is a diverse genus in the legume family. This genus contains both annual and perennial species, mostly herbaceous, but some shrubby and tree types also exist.

Lupins have a long history of being used both as ornamental plants in gardens and as an agricultural crop. Four lupin species, *L. angustifolius*, *L. albus*, *L. luteus* and *L. mutabilis*, have gained agricultural importance. Australia is the largest lupin grain producer in the world and *L. angustifolius* is the dominant lupin species for lupin production in Australia. It is used mainly as animal feed, and to a lesser extent, for human consumption in some European and South American countries. Lupin seeds are currently receiving increasing international interest as an alternative source of human food ingredients due to its high quality protein and dietary fibre.

SECTION 1 TAXONOMY

Lupinus is a large and diverse genus in the legume family (Fabaceae). Its common name used in Europe and Australia is lupin for both native and domesticated species, while the common name for native *Lupinus* in North America is lupine (Information portal for lupins 2010a). Taxonomically, lupins are classified within order Fabales, family Fabaceae, tribe Genisteae and genus *Lupinus L.* (Clements et al. 2005a). The number of species in this genus is not well defined and it was thought to be over 1000 (Kurlovich et al. 2002b). However, the commonly agreed number of the existing lupin species is around 280 (Eastwood et al. 2008). At present, the number of accepted *Lupinus* species recorded in the Integrated Taxonomic Information System (<http://www.itis.gov>) is 164.

The *Lupinus* genus contains both annual and perennial herbaceous species, and some shrubby and tree types (Ainouche & Bayer 1999). Its rich diversity of species has also been grouped into Mediterranean and North African ‘Old World’ species and American ‘New World’ species, covering a wide climate range.

The number of Old World species is limited, represented by only 12 annual species. These have been divided into two distinct groups, Malacospermae and Scabrispermae, primarily based on seed coat texture: the smooth-seeded and the rough-seeded species, respectively (Gladstones 1984). The Malacospermae group consists of five smooth-seeded species: *L. angustifolius*, *L. albus*, *L. luteus*, *L. hispanicus* and *L. micranthus*, and they are distributed around the Mediterranean and exhibit variable chromosome numbers ranging from $2n = 40$ to 52 (Naganowska et al. 2003; Wink et al. 1999). The Scabrispermae group comprises seven rough-seeded species: *L. pilosus*, *L. cosentinii*, *L. digitatus*, *L. princei*, *L. palaestinus*, *L. atlanticus* and *L. somaliensis*. These species are mainly distributed in North Africa and in the Eastern part of the Mediterranean region with chromosome numbers ranging from $2n = 32$ to 42 (Naganowska et al. 2003; Wink et al. 1999).

The New World *Lupinus* is taxonomically difficult with species poorly defined. It has been proposed that there are about 500 taxa in the New World with over 1700 species names suggested (Dunn 1984). More recent evidence suggests that the New World lupins may be treated as a broadly defined polymorphic species (Ainouche & Bayer 1999). The base chromosomal number suggested for this group is $x = 6$ and they are regarded as paleopolyploids¹ that behave as diploids (Dunn 1984). Most of the New World species cytologically investigated, including *L. mutabilis*, display a common chromosome number of $2n = 48$ with some occasional individuals having $2n = 36$ and 96 (Ainouche & Bayer 1999; Camillo et al. 2006).

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

Lupin serves as a fodder and food crop, as well as an ornamental plant. Some species have been bred to enhance their ornamental value, while others have been a traditional food in the Mediterranean region and the Andean highlands in South America. In Australia, a modern farming system based on wheat:lupin rotation has been in place for over 40 years (DAFWA 2010).

This diverse genus exists in both the Eastern and Western Hemispheres. The centre of origin for this genus has not been determined and three different centres of origin have been proposed that include the Mediterranean, North America and South America (Hondelmann 1984; Kurlovich et al. 2002b). Molecular evolution studies suggest that the centre of origin is the Mediterranean and northern and eastern African region for the Old World species, and two lineages lead to the New World species in North America and South America, respectively (Wink et al. 1999; Wolko et al. 2011).

Approximately 90% of the recognised lupin species are distributed in temperate and subtropical zones of North and South America, ranging from Washington State of the USA to southern Argentina and Chile. The remaining species are distributed in the Mediterranean region and Africa, with some populations extending to highland and mountain regions of tropical East Africa and the subarctic climate of Alaska and Iceland (Gladstones 1998; Wolko et al. 2011). The geographical distribution of the major lupin species is shown in Appendix 1.

Lupins have an ancient history in agriculture that traces back more than 4000 years (Kurlovich 2002). Domestication occurred first in the Mediterranean region and the American continent, but the real breakthrough that made lupin a modern agricultural crop occurred in Europe and Australia. The history of lupin domestication may be outlined as follows (Clements et al. 2005a; Kurlovich 2002):

- Before 2000 BC. Primary domestication of *L. albus* in ancient Greece and Egypt to produce grain for human and animal consumption, as well as for cosmetics and medicine
- 1000-800 BC. Utilization of *L. albus* as green manure in ancient Rome and, subsequently, in other Mediterranean countries

¹ A paleopolyploid is a eukaryote in which ancient genome duplications have occurred. As a general consensus, many of the flowering plants are paleopolyploids (Blanc & Wolfe 2004).

- 700-600 BC. Primary domestication of Andean pearl lupin (*L. mutabilis*) on the American continent
- 1860s. Domestication of *L. luteus* and *L. angustifolius* for green manure production in Baltic countries and afterwards in Germany
- 1927-1928. Methods for selecting low alkaloid lupin mutants developed in Germany
- 1930s-1970s. Sweet lupin varieties with permeable seeds were developed from *L. luteus*, *L. albus*, *L. angustifolius* and *L. mutabilis* in Germany, Sweden and Russia
- 1980s-1990s. Fully domesticated *L. cosentinii* and further domestication of other potential lupin species (*L. atlanticus* and *L. pilosus* and *L. polyphyllus* Lindl.) in Australia and Russia.

In their wild state, lupins have ‘hard’ (water impermeable) seeds, shattering pods and high level of alkaloids that makes lupin seeds toxic for human and animal consumption. The breakthrough in selecting natural mutants in *L. luteus* with low alkaloids (sweet type) by Von Sengbusch in Germany in 1927/1928, after the development of a quick method for detecting alkaloids, opened a new era in modern lupin breeding (Hondelmann 1984). Modern lupin breeding has focused on developing lupin species/varieties which produce seeds that are sweet and water-permeable, and non-shattering pods to facilitate mechanical harvest.

Domesticated lupin species have been grown as a cultivated crop in many countries on five continents. Most of the agriculturally important species are the Old World species due to their larger seed size and well-formed embryo. The Mediterranean lupin forms are characterized by a sympodial type of branching (tending to have lateral growth) and are mainly self-pollinated (Kurlovich 2002). Among them, smooth-seeded *L. angustifolius*, *L. albus* and *L. luteus* have been widely included in agricultural practice in many countries including Australia. In addition, rough-seeded *L. cosentinii* Guss. has been domesticated in WA (Cowling & Gladstones 2000). Some developmental work on *L. atlanticus* and *L. pilosus* has been done in Australia to suit production on calcareous (alkaline) soils (Brand et al. 2002; Buirchell & Cowling 1992; Miao et al. 2001).

The New World lupins are less specialized than the Old World ones. They are generally characterized by a more primitive monopodial type of branching (tending to grow upward from a single point to form a single stem) and by the cross-pollination habit (Kurlovich 2002). Their seeds are small with differentiated embryo and generally have little endosperm, making them unattractive for grain production. Among the New World species, only *L. mutabilis* (pearl lupin) is domesticated and cultivated as a food crop throughout the Andes (Eastwood & Hughes 2008). Another species *L. polyphyllus* (Washington lupin) is present in many countries as a weed and the effort to turn it into a domesticated fodder crop is still on-going (Kurlovich et al. 2008).

2.2 Commercial uses

Lupins are valuable not only as garden ornamentals, but also as an agricultural crop with increasing importance for various agricultural and aquacultural uses. Many varieties and hybrids of lupins, such as Russell lupin (*L. polyphyllus*) and Rainbow lupin (*L. regalis*), have long been used as garden flowers due to the variety of colours and showy nature of the tall flower spikes.

Like other members in the legume family, lupins fix atmospheric nitrogen through rhizobium-root nodule symbiosis and convert it to a usable form that improves soil quality. Therefore,

they are tolerant to infertile soils and have long been incorporated in agricultural practice as green manure and in rotation with other crops. For instance, the wheat:lupin rotation has been used as a crop production system in Western Australia (WA) for over 40 years and sustained wheat yields are directly dependent on the rotational benefits of lupins (DAFWA 2010). In addition, lupin stubble residues are a very nutritious livestock fodder for grazing.

However, the commercial value of lupins comes mainly from the lupin seed. The majority of the world's lupins are used for stockfeed. Ruminants (such as cattle and sheep) are the biggest animal consumer group followed by pigs and poultry (Information portal for lupins 2010c). Lupins are often used as a substitute for high-protein soybean meal in livestock feeds. The low levels of starch and high levels of fermentable carbohydrate make lupins a desirable feed for ruminants. Australia, Europe and Japan use sweet lupins in dairy production. In Australia, the largest utilisation of lupins is whole grain feeding to sheep, to supplement low grade roughage diets (Lawrance 2007). The hull of lupins is a readily digestible fibre for ruminants, while the lupin kernel can be used directly as monogastric feeds.

There is increasing demand for lupin grain in aquaculture due to the superior digestibility of lupin proteins (Glencross 2005; Glencross 2001). The aquaculture industry uses lupin seed and kernel meal as a feed to replace high protein fish meal. Salmonid and prawn are the two key aquaculture feed markets and lupin kernel meal is used widely in feed formulations. Up to 40% of lupin can be included in the fish meal for rainbow trout without palatability and growth problems (Glencross 2008).

Lupins also have a long history of being consumed by humans in the Mediterranean and Andean regions (Petterson 1998). However, less than 4% of global lupin production is used as human food (Lawrance 2007). Lupin seeds possess many nutritional and food processing qualities, making them an attractive alternative to dry beans and soybeans. Foods derived from lupins are commercially manufactured in Europe, North America and Australia. These include lupin kernel flour based products such as bread, pasta, milk, tofu, tempe, miso, soy sauce and snack foods. Also lupin hull is used as dietary fibre products or fibre additive to bread (Information portal for lupins 2010d; Petterson 1998).

Australia is currently the biggest lupin supplier in the world. An averaged 41% of annual Australian lupin production was exported during the five years to 2005-06. Over this period, exports averaged around 430,000 tonnes, with a value of nearly \$100 million, a year (Lawrance 2007). This accounts for around 2% of the total value and volume of Australian exports of grains and oilseeds. In 2007, the main destinations for Australia's lupin exports were the South Korea, European Union, Japan and Chinese Taipei with each export destination taking around 50, 27, 12 and 3%, respectively (Lawrance 2007). A recent figure shows that South Korea, Japan, Netherland, Malaysia and Germany were the top five Western Australian lupin export markets in 2010-11 (DAFWA 2012).

2.3 Cultivation in Australia

Lupins were first introduced into Australia in the mid-19th century. At the end the century, lupins were being used as a fodder crop for animal feed. After the discovery of the usefulness of leguminous crops in fixing atmospheric nitrogen, lupins were also grown as green manure. Under Australian conditions, natural nodulation of lupins by soil bacterium *Bradyrhizobium* is generally poor and inoculants containing selected strains of bradyrhizobia need to be applied

with the seed, or in the planting furrow, to ensure adequate nodulation (Information portal for lupins 2010a).

Modern lupin production as a grain legume in Australia did not begin until the first fully domesticated cultivar of *L. angustifolius*, Uniwhite, was released in WA in 1967 (Cowling et al. 1998). After that, many new varieties from different lupin species with improved flowering time, adaptation, yield and resistance to diseases were developed, which made Australian lupin production into a profitable industry. The major species for lupin production in Australia are *L. angustifolius* (narrow leafed lupin), *L. albus* (white lupin) and *L. luteus* (yellow lupin). The lupin varieties recommended for growing in Australia are provided in Appendix 2.

L. angustifolius is the most important species in Australia, comprising over 95% of all lupin grain production in WA (DAFWA 2010). In the wild state, *L. angustifolius* has blue flowers that produce bitter seeds and is referred to as ‘blue lupin’. However, all cultivated varieties of this species have been bred to have white flowers that set seeds with low alkaloids to distinguish them from their bitter wild relatives. These varieties are named ‘narrow-leafed lupin’ by researchers in Australia but the name ‘Australian Sweet Lupin’ is often used by the industry (Information portal for lupins 2010a). To avoid confusion in this document, the scientific name *L. angustifolius* will be used in the subsequent sections to mean the domesticated narrow-leafed lupin, with its wild counterpart noted otherwise.

Most Australian lupin production occurs in the winter/spring rain-fed parts of south-western WA, followed by South Australia (SA), southern New south Wales (NSW) and Victoria. Lupins are generally sown in autumn (between late April and early June) and harvest occurs in October and November with terminal drought determining crop ripening. Production in WA and SA is dominated by *L. angustifolius*. There is a significant proportion of *L. albus* produced in NSW and Victoria. Albus lupin production has been steadily increasing in recent years from around 15,000 hectares in 2005 to exceed 30,000 hectares in 2009 with export earnings to growers exceeding \$15 million (Bray 2010). Figures for lupin production in Australia are shown in Table 1.

Table 1. Lupin growing area and production in Australia^a

State	Area planted ('000 ha) (A) and Production (kt) (P)	Five year average ^b	2008-09	2009-10	2010-11	2011-12 ^c
Western Australia	A	618	444	500	522	503
	P	753	600	631	398	779
South Australia	A	79	53	53	64	65
	P	82	52	74	93	74
New South Wales	A	65	44	102	128	76
	P	44	37	84	252	80
Victoria	A	36	36	36	42	46
	P	26	18	33	65	43
Total	A	798	577	691	756	690
	P	906	707	822	808	976

a, Source: (ABARE 2010; ABARES 2011; ABARES 2012a; ABARES 2012b); b, Five years to 2007-08; c, ABARE estimate

2.3.1 Commercial propagation

Although the varieties for lupin grain production are primarily self-pollinated, they still readily cross with the aid of insects such as bees. This is particularly true for sweet albus lupin (Richards 2010). Isolation is therefore absolutely necessary to maintain purity during seed propagation. According to the South Australian Seed Certification Scheme (Smith & Baxter 2002), for *L. albus* and *L. luteus*, the isolation for producing basic seed² and certified seed³ are 100 m and 50 m from other cultivars, respectively. While for *L. angustifolius*, the isolation for both basic seed and certified seed is 3 m. However, the OECD Seed Schemes (OECD 2008) require the isolation to be 200 m for fields of 2 ha or less and 100 m for fields larger than 2 ha for all legume crops including these three lupin species.

In Australia, many lupin varieties are covered by Plant Breeder's Rights (PBR) (Wheeler & McCormack 2010). Seed of registered varieties cannot be sold, traded or given away without the authorisation of the rights owner or licensee. In addition, seed royalties are charged to growers for using a PBR-protected variety. A system of end point royalties (EPR) developed by the breeding community, the Grains Research and Development Corporation (GRDC) and the seed industry are increasingly used by breeders and marketing agents (GRDC 2008; Wheeler & McCormack 2010). The PBR information and marketing agents for lupin varieties in Australia can be found in Appendix 2.

Seed certification is available through Seed Service Australia,ASUREQuality Australia Ltd, AGWEST Plant Laboratories and QSEED Pty Ltd on a voluntary basis. For Sweet albus lupin seed, traders also participate in an ultraviolet testing scheme to ensure that all seed lots are free of bitter seed contamination (Richards 2010).

2.3.2 Scale of cultivation

Australia has been the dominant lupin producer in the world since 1990. It accounted for around 85% of global lupin production over the ten years to 2006 (Lawrance 2007). From 2006, Australian lupin production reduced dramatically due to drought. In 2008, the global lupin production was around 774,000 tonnes, of which 63% was produced in Australia (FAO 2008). Other major lupin producers include Belarus, Poland, Germany, Chile and Russia.

According to the 2012 figure from Australian Bureau of Statistics, in terms of growing area lupins are the fifth largest crop grown in Australia after wheat, barley, canola and oats. The area sown to lupins peaked at 1,425,000 ha in 1997 and production reached the peak of 1,968,000 tonnes in 1999. However, the area harvested dropped to 500,000 ha in 2006 mainly due to drought (FAO 2008) and has since remained around this level (Table 1). WA is the largest lupin producing state, which accounts for an average 77% of total Australian production in the eight years from 2003 to 2011 (Table 1). *L. angustifolius* has been the dominant species in the production system in WA due to its adaptability to sandy and acidic soils and the Mediterranean climate of south-western Australia. The varieties of *L. angustifolius* listed in Appendix 2 have all been grown in WA. Gungurru and Merrit were the most popular varieties grown in all districts in WA for a number of years (French &

² Basic seed is derived from areas sown with pre-basic seed (derived from breeders seed) and produced under the supervision of the breeder and the certification authority.

³ Certified seed is derived from basic seed.

D'Antuono 2003), but have now been displaced by higher yielding/better disease or pest resistance varieties such as Quilinoch, Mandelup and Jenabillup (Wheeler & McCormack 2010).

NSW and SA are the next largest lupin producing states followed Victoria (Table 1). In SA, the *L. angustifolius* varieties also dominate lupin production with Mandelup the variety of choice for 2009 (Egan & Crouch 2009). In NSW, the production of sweet albus lupin has steadily increased since an outbreak of the fungal disease anthracnose in WA in 1996 stopped albus lupin production in WA. Sweet albus lupin generally has higher yield potential than *L. angustifolius* lupins and the new varieties such as Luxor and Rosetta are free of bitter seed contamination (Smith 1999; Viterra Seeds 2008). Sweet albus lupin is ideal for human consumption, with Egypt the main export destination. The ideal areas for growing albus lupins are the medium to high rainfall zones around the region of Albury, Wagga Wagga, Young and Cowra. Ideal conditions also exist around Gilgandra and Coonamble in central NSW (Penfold 2006).

2.3.3 Cultivation practices

In Australia, lupin is generally grown in areas receiving less than 500 mm annual rainfall (French & Buirchell 2005). They are sown between late April and early June and the optimal sowing times depend on the rainfall zones and soil types. As a general guide, the sowing times on sandy soils are mid-April to early May, early to mid-May and mid-May for zones with yearly rainfall below 350 mm, 350 to 450 mm and above 450 mm, respectively. While on sandy loams and loam soils, the sowing time can be delayed to late May or early June for zones receiving yearly rainfall above 450 mm (DPI Victoria 2010). Cultivated lupins begin flowering from late July to early September and are harvested in October or November.

In general, lupins grow well on soils that are well drained, friable with reasonable depth and slightly acidic or neutral. The sensitivity of various cultivated lupin species to soil pH, waterlogging (saturation of the soil by water) and soil fertility varies (Table 2). *L. angustifolius* adapts well to acidic sandy soils with low fertility and is resistant to transient waterlogging. In contrast, *L. albus* prefers fertile soils with high pH and is sensitive to waterlogging.

Table 2. Adaptation of cultivated lupin species to different soil types*

Soil factor	Least adapted	Less adapted	Adapted	Most adapted
Low pH (high Al)	<i>L. albus</i>		<i>L. angustifolius</i>	<i>L. luteus</i>
High pH (high HCO ₃ ⁻)	<i>L. angustifolius</i>	<i>L. luteus</i>	<i>L. albus</i>	<i>L. pilosus</i> <i>L. atlanticus</i>
Transient waterlogging	<i>L. albus</i>	<i>L. atlanticus</i>	<i>L. angustifolius</i>	<i>L. luteus</i>
Low fertility (sandy soils)	<i>L. albus</i>		<i>L. angustifolius</i>	<i>L. luteus</i>

*Sources: (Brand et al. 2002; Information portal for lupins 2010b)

Timely harvest is important to maximise grain quality and prevent yield loss. In general, lupin crops are harvested within three weeks after most seeds reach physiological maturity, a stage at which the seed-filling period has ended and the seed has reached its maximum dry weight

(Walker et al. 2011). Delays can result in significant yield losses because of lodging, pod shattering and pod drop. Harvest should start as soon as the moisture content reaches 14% (Riethmuller 2008). Harvesting when humidity is high (eg early morning or at night) can substantially reduce seed loss by shattering or pod drop. Windrowing is a useful harvest method for lupin (Carpenter 2000).

Lupin crops are often grown in rotation with other crops, usually cereals. Incorporating lupin into rotations confers benefits to the farming system that include reduced disease in the subsequent cereal crop, increased supply of organic nitrogen, increased supply of high quality sheep feed and more options to control weeds. In WA, lupins are an integral part of the farming system (DAFWA 2010). Wheat:lupin rotations widely used in the 1980s and 1990s contributed directly to increased wheat yield and effective control of weeds. However, with an increase in resistance of weeds to many of the herbicides commonly used in the wheat:lupin rotation, farmers have begun to incorporate a greater range of crops in their rotations (Harries & Peek 2008). A recent report showed that lupin-wheat-barley rotation is currently one of the most profitable farming system options (Baxter 2010).

2.4 Crop improvement

Lupins can be improved through conventional breeding based on natural germplasm stocks and genetic engineering may play an important role in future lupin crop improvement. The countries with significant breeding programs include Australia, Poland, Russia, Germany, Belarus and Chile. Other countries including the USA, Denmark, Spain, Portugal and Iceland have smaller breeding programs (Clements et al. 2012).

After the release of the first *L. angustifolius* variety, Uniwhite, breeding for improved agronomic characteristics, particularly yield and disease resistance, has continued in Australia. *L. angustifolius* breeding has been the major focus particularly in WA and SA, but sweet albus lupin breeding has attracted more interest in NSW. Similar breeding work was done in Chile, Germany, Poland and Russia. In Europe and Russia, breeding programs have mainly been targeting *L. albus* and *L. luteus* (Information portal for lupins 2010b; Kurlovich & Kartuzova 2002).

2.4.1 Breeding

Modern lupin breeding relies on genetic material from wild lupins, and both natural and induced mutants (Cowling et al. 1998). The basic lupin breeding method is the typical step-by-step intraspecific hybridisation. Multiple, back, reciprocal, diallelic and polyallelic crossings are used in recurrent schemes of hybridisation. Due to reproductive barriers, interspecific crossing between the Old World and the New World species cannot produce fertile hybrids under natural conditions (Kurlovich & Kartuzova 2002). Although viable F1 or F2 seeds or plants have been produced from crosses among the Old World species or the New World species (Clements et al. 2008; Gupta et al. 1996), no such material has so far been successfully used in any commercial breeding program. However, flowering F1 hybrid plants between *L. angustifolius* and *L. luteus* have recently been obtained. These plants showed intermediate morphological characteristics and their true hybrid status has been confirmed by molecular marker analysis (Clements et al. 2009a). Backcrossing these hybrids to certain *L. angustifolius* cultivars may generate novel *L. angustifolius* varieties with desirable characteristics, such as superior seed quality, from *L. luteus*.

The majority of early lupin cultivars were produced with the use of spontaneous or induced mutants. For *L. angustifolius*, breeding has involved the introduction of key domestication traits controlled by mutations at five or six loci (Nelson et al. 2006) and these alleles are recessive. The *Iucundis* (*Iuc*) allele controls alkaloid production and bitterness and the recessive mutant *iuc* was exploited to produce “sweet” low alkaloid forms (Gladstones 1977). *Mollis* (*Moll*) controls water permeability of seed, “hard” seeds being important for long term survival of the species in the wild, but the recessive mutant *moll* is necessary to allow immediate germination upon sowing (Mikolajczyk 1966). Two genes are known to be responsible for pod shattering, *Tardus* (*Ta*) and *Lentus* (*Le*), and the additive effect of the recessive mutants *ta* and *le* prevents pod shattering at harvest (Gladstones 1967). Early flowering is promoted by the dominant mutant allele *Ku*, which is important for adaptation to short growing seasons in Australia (Gladstones 1977). *Leucospermus* (*Leuc*) controls pigment production in seeds, cotyledons, and flowers and the recessive mutant *leuc* has been used to differentiate the domesticated crop by its white flowers and seeds from the bitter, blue-flowered, darkseeded wild populations which may grow in the same region (Gladstones 1977).

Mutagenesis has long been incorporated in lupin breeding. The common mutagens used in lupin breeding include ionizing radiation (X-ray and gamma rays) and chemical agents including EI (ethylene imine), EMS (ethyl methanesulphonate), NMH (nitroso methyl urea) and DMS (dimethyl sulphonate) (Mutant Varieties Database at <http://www.docstoc.com/docs/15258973/Mutant-Varieties-Database>). In Ukraine, various mutants were generated through irradiation in *L. albus* and used in breeding programs for the generation of alkaloidless varieties such as Kiev Mutant (Golovchenko 1982). In Australia, X-ray mutagenesis was used to produce genes for low alkaloid content (*sw*), early flowering (*xe*) and white flowers (*wfs*) in *L. cosentinii*; and the early flowering gene was induced in *L. angustifolius* by EI (Cowling et al. 1998). At the Centre for Legumes in Mediterranean Agriculture in WA, two *L. angustifolius* mutants highly resistant to metribuzin (Tanil-AZ-33 and Tanil-AZ-55) were recently created by treating seeds with sodium azide (Si et al. 2009).

The targeted traits for more recent breeding programs include yield, resistance to diseases and abiotic stress, biochemical structure associated with seed quality, nitrogen fixing ability, duration of vegetation, plant architecture and non-dehiscent pods (Cowling et al. 1998; Kurlovich & Kartuzova 2002). For large scale selection of these targeted traits, molecular breeding has attracted more attention and funding. A genetic linkage map based on microsatellite-anchored fragment length polymorphism (MFLP) (Boersma et al. 2005) and a gene-based linkage map (Nelson et al. 2006) have been developed in *L. angustifolius*. In addition, a linkage map of *L. albus* combining amplified fragment length polymorphism (AFLP) and gene-based markers has also been developed (Phan et al. 2007). Large scale marker-assisted selection for various traits of industry importance has been utilised in lupin breeding. For instance, molecular markers tagging anthracnose resistance and phomopsis stem blight resistance in *L. angustifolius* and *L. albus* have been developed and applied in breeding programs in Australia (Yang et al. 2010; Yang et al. 2008; You et al. 2005).

2.4.2 Genetic modification

Currently, there is no report of commercial production of genetically modified lupin species (Eapen 2008; Information portal for lupins 2010b). However, research on genetic engineering of lupins has been carried out in countries such as Australia, Poland and the USA. The

purposes for generating GM lupins vary and include scientific research, crop improvement and using lupin as a bioreactor for producing proteins of medicinal importance.

So far, gene transfer to lupin has all been conducted via *Agrobacterium*-mediated transformation. Stable lupin transformation has been achieved using strains from either *A. tumefaciens* or *A. rhizogenes*. Target lupin species used for genetic engineering have included *L. angustifolius*, *L. albus*, *L. luteus* and *L. mutabilis*. Detailed information in relation to lupin transformation is outlined in Table 3.

Table 3. Lupin transformation

Institution	Lupin species	Agrobacterium species/Strain	Explant	Selectable marker	Transgene of interest	Reference
Florigene Pty Ltd & University of Western Australia	<i>L. angustifolius</i>	<i>A. tumefaciens</i> /AGL0, LBA4404, EHA101	Shoot apices	<i>bar</i>		(Pigeaire et al. 1997)
Murdoch University	<i>L. luteus</i> ; <i>L. angustifolius</i>	<i>A. tumefaciens</i> /AGL0	Hypocotyl and radicle	<i>bar</i>	<i>Nla</i> ; <i>Nlb</i>	(Li et al. 2000); (Jones et al. 2008)
CSIRO	<i>L. angustifolius</i>	<i>A. tumefaciens</i> /AGL0	Embryonic axis; shoot apices	<i>bar</i>	<i>ssa</i> ; <i>Atlg55920</i>	(Molvig et al. 1997); (Tabe et al. 2010)
University of Western Australia	<i>L. angustifolius</i>	<i>A. tumefaciens</i> /AGL0	Root	<i>bar</i>	<i>p35</i>	(Wijayanto et al. 2009)
	<i>L. angustifolius</i>	<i>A. tumefaciens</i>	Shoot apices	<i>bar</i>	<i>ipt</i>	(Atkins et al. 2011)
University of Minnesota, USA	<i>L. albus</i>	<i>A. rhizogenes</i> /A4TC24	Radicle	<i>nptII</i>		(Uhde-Stone et al. 2005)
Institute of Bioorganic Chemistry, Poland	<i>L. luteus</i>	<i>A. tumefaciens</i> /C58	Cotyledon	<i>nptII</i>		(Kapusta et al. 1999)
Institute of Plant Genetics, Poland	<i>L. luteus</i>	<i>A. tumefaciens</i> /LBA4404, GV3101, EHA105, C58, A281, Ach5	Hypocotyl	<i>nptII</i>	<i>HBsAg</i>	(Pniewski et al. 2006)
University of Nottingham, UK	<i>L. mutabilis</i>	<i>A. tumefaciens</i> /LBA4404; <i>A. rhizogenes</i> /R1601	Shoot apices; hypocotyl & epicotyl	<i>nptII</i>		(Babaoglu et al. 2004; Babaoglu et al. 2000)

In Australia, genetic modification of lupins is mainly focused on generating lines with enhanced seed protein profiles, herbicide tolerance and disease resistance directly associated with lupin seed quality and yield. Researchers at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) have made attempts to increase sulfur accumulation in lupin seeds by introducing a chimeric sunflower seed albumin (*ssa*) gene into *L. angustifolius* (Molvig et al. 1997). The sunflower seed albumin protein is sulfur-rich and contains 16% methionine and 8% cysteine. Expression of this gene in GM lupin seeds increased methionine but not cysteine levels (Tabe & Droux 2002). A gene coding for the serine acetyltransferase (SAT) from *Arabidopsis thaliana* was also introduced into *L. angustifolius*, resulting in a

dramatic increase of free cysteine in developing seeds (Tabe et al. 2010). However, increasing the total sulfur composition in mature GM seeds has not been achieved.

GM *L. angustifolius* were also generated by the introduction of a nuclear inclusion protein b gene (*Nlb*) from the Bean yellow mosaic virus (BYMV) (Jones et al. 2008). The aim of this work was to increase the resistance of *L. angustifolius* to BYMV. However, no GM lines displayed improved resistance, probably due to gene silencing. GM *L. angustifolius* plants containing the isopentenyl pyrophosphate transferase gene (*ipt*) were also produced in an attempt to increase pod set and grain yield (Atkins et al. 2011).

Some GM lupins have been trialled in Australia. University of Western Australia has conducted field trials of *L. angustifolius* and *L. luteus* genetically modified for resistance to the herbicide Basta and Bean Yellow Mosaic Virus (OGTR 2001). CSIRO has also carried out field trials of GM high sulfur lupins (*L. angustifolius*) (GMAC 1998), but these lines have not been commercialised (Smith & Atkins 2008).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

As described in Section 1, *Lupinus* is a genus with diverse species. Some of the species are annual plants (e.g. those Old World species of agricultural significance), while most species are herbaceous perennial plants and a few are shrubs. Plant height of various species ranges from 0.2 -1.5 metres with some shrubs reaching 2.5 metres. Only a brief description of the morphology and anatomy of lupin is presented here with an emphasis on herbaceous annual species. An example of different parts of a lupin plant is shown in Figure 1.

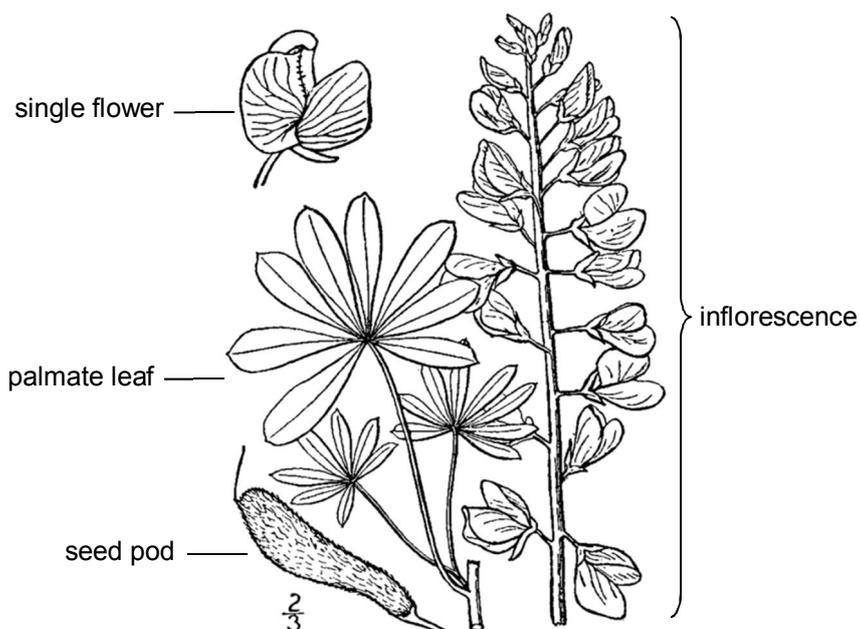


Figure 1. An illustration of different parts of *L. perennis* (modified from: USDA-NRCS PLANTS Database / Britton, N.L., and A. Brown. 1913. *An illustrated flora of the northern United States, Canada and the British Possessions*. Vol. 2: 348) (Britton & Brown 1913).

3.1.1 Root

Lupins generally have a taproot system. Root morphology varies widely between species, ranging from a dominant taproot with relatively few lateral roots to a highly developed lateral root system (Clements et al. 1993). For lupin species with a tap root system, the main root reaches the depth of 1-2 metres. Lupin roots, especially the main axis, bear nodules formed by *Bradyrhizobium* for nitrogen fixation. In addition, morphological adaptations occur in many plants for increased nutrient uptake. For instance, proteoid roots, also known as cluster roots, can form in response to phosphorus or iron deficiency (Gardner et al. 1982; Gilbert et al. 2000; White & Robson 1989a). Root morphologies may reflect differences in the adaptation of lupine species to different soil types. In the case of domesticated genotypes of *L. angustifolius*, which are well suited to deep sandy soils, the plants have a dominant taproot and a high number of primary lateral roots, but relatively few secondary or tertiary lateral roots, with no proteoid root formation (Clements et al. 1993).

3.1.2 Stem

Lupin stems vary among species and are fascicular for herbaceous species and arborescent (treelike) for shrub species (Kurlovich et al. 2002b). The cross-section of lupin stem is commonly terete shape. Annual lupin species differ from each other by the shape of the cross-section of their stems and by size (Petrova 2002). The surface of lupin stems is either pubescent with various degree of density or naked with a waxen tinge.

3.1.3 Leaf

Lupins have a characteristic palmate leaf shape with leaf blades divided into various numbers of leaflets. The shape of leaflets varies largely among different species, including oval oblong, ovate oblong, obovate, narrow linear, calceolate and more (Kurlovich et al. 2002b). The surface of leaflets is in most cases covered by silver three-celled hairs with various densities (Petrova 2002). Leaves are soft green or greyish green and connected to stems by long petioles (leafstalks) with elongated stipules.

3.2 Reproductive morphology

In the majority of lupin species, the main stem and lateral branches terminate into racemes of the apical truss type (Figure 1). This type of inflorescence has an ascending flowering order and flowers are produced in dense or open whorls on an erect spike with the bottom flowers blossoming first. The flower is hermaphroditic. It is zygomorphous (bi-laterally symmetrical) with a typical pea flower shape 1-2 cm long, consisting of five joined sepals, five petals, an ovary with a pistil and ten stamens (Figure 2). The petals are not all joined and are of different shapes and sizes. The uppermost petal is called the standard (also called the vexillum or flag) and the two partly joined petals at the side are the wings. Within the wings are two partly joined petals forming a boat-shaped keel (carina). Inside the keel are the long, narrow and pod-shaped ovary and ten concrescent stamens arranged in two circles of five each. The ovary usually contains two or more ovules.

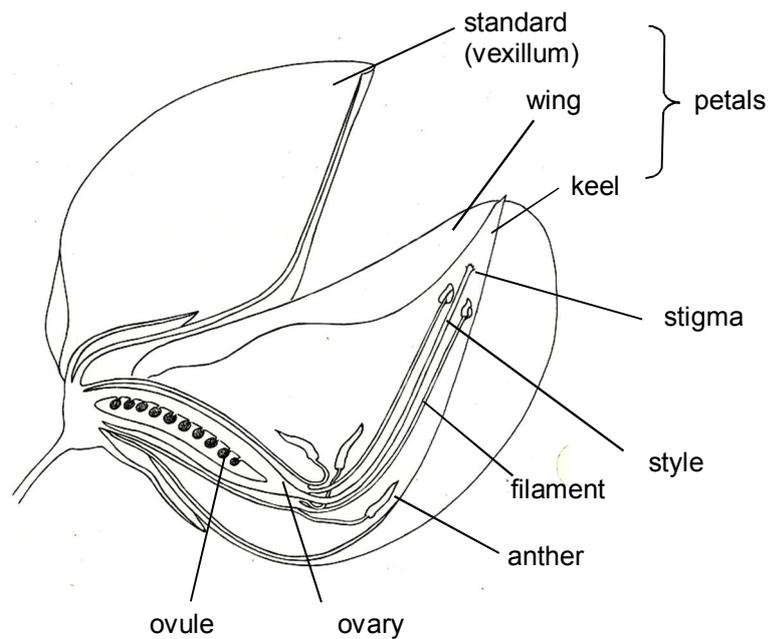


Figure 2. Structure of a lupin flower (*L. perennis*) – half flower (© D G Mackean)

The lupin pod is orbicular or flattened in a cross-section view and straight or curved longitudinally. The pod surface is rough and pod colour varies from cream, brown to black. Some species have easy shattering pods while others have non-shattering or weakly shattering pods.

Lupin seeds are very diverse in size, shape and colour and their surface can be smooth or rough. The seed stalk hangs over the micropyle. Within the seed, the bent embryo is at the top of the cotyledon where nutrients are stored. Primary true leaves are opposite, while other leaves cannot be seen until germination.

SECTION 4 DEVELOPMENT

4.1 Reproduction

Lupins can reproduce both sexually and vegetatively. Under natural conditions, most annual lupin species are self-compatible and mainly reproduce by self-pollination. For example, *L. angustifolius* is almost exclusively self-pollinated (Kazimierska & Kazimierski 2002). In contrast, perennial lupin species reproduce mainly through cross-pollination due to self-incompatibility (Kittelson & Maron 2000; Kurlovich 2002). Asexual reproduction is only common through vegetative regeneration in perennial lupin species. There is no evidence to show that lupin can reproduce through apomixis (Richards 1986).

4.1.1 Asexual reproduction

For the annual lupin species commonly used in agricultural practice, no vegetative reproduction has been reported. However, under natural conditions, some perennial lupin species reproduce vegetatively. For example, broadleaf lupin (*L. latifolius*) can reproduce

from root sprouts, root fragments, and root caudex (Reeves 2010). Garden lupin (*L. polyphyllus*) can spread by means of creeping rhizomes below ground (Fremstad 2006). For many ornamental perennial species, such as *L. polyphyllus*, basal cuttings and divisions are used for propagation. More colourful perennial hybrids of ornamental species can be maintained and produced vegetatively to ensure the production of plants with same coloured flowers.

4.1.2 Sexual reproduction

All lupin species reproduce sexually by producing seeds. They produce an inflorescence in the form of a spike (raceme) of the apical truss type (see Section 3.2). Flowering on the main inflorescence (primary flower set) in Old World lupins starts 59-136 days from planting depending on species, genotypes and the growth conditions (Buirchell & Cowling 1998). The most basal flower on the inflorescence is the first to reach anthesis. The secondary flower set on branches, with *L. angustifolius* and *L. albus* as examples, reach anthesis in about 10-15 days after primary flower setting and then tertiary flower set follows in about the same number of days (Dracup & Kirby 1996b; French & Buirchell 2005). Typically, around 30 flowers may open on a main shoot inflorescence, lasting about 20 days, and branches bear fewer flowers and the flowering duration is shorter (Dracup & Kirby 1996a).

In Australia, lupin is normally sown in autumn and starts flowering in spring (mid-August to early September). Time to flower from seeding varies among species and may be influenced by vernalisation and photoperiod. Variation in vernalisation requirements and response in various lupin species and their genotypes has been reported (Adhikari et al. 2008; Clapham & Willcott 1995; Landers 1995; Putnam et al. 1993; Rahman & Gladstones 1974; Reader et al. 1995).

Different genotypes within one species may have varied response to vernalisation. Within *L. albus*, there are three types (also called morphotypes): winter, semi-winter and spring. Winter types have an obligate requirement to be vernalised (cold treatment) to complete their life cycle; semi-winter types flower without cold treatment, but only after prolonged vegetative growth. Spring types are similar to semi-winter types and flower without cold treatment, but cold treatment can shorten the time from vegetative growth to floral differentiation (Clapham & Willcott 1995). In *L. angustifolius*, there are three types of response to vernalisation: an absolute requirement; a reduced response, in which vernalisation does not appear to be essential for flowering; and no response in modern varieties carrying a dominant early flowering gene *Ku* (Landers 1995). Most modern lupin varieties used in Australia do not have a vernalisation requirement for flowering, although the early varieties grown in WA do require vernalisation (French & White 2008).

The timing of flowering is also controlled by photoperiod (or daylength) to various degrees for various lupin species and their genotypes. Flowering is generally hastened by long days and reduction of photoperiod can retard initiation (Dracup et al. 1998c; Rahman & Gladstones 1974). However, the rates and duration of seed filling have large variation between genotypes and physiological maturity does not necessarily correlate with flowering time (Dracup et al. 1998c).

Sensitivity to photoperiod differs remarkably among different species. Rahman and Gladstones (1974) showed that *L. luteus* was the most sensitive species followed by *L. cosentinii*, *L. angustifolius* and *L. albus* under artificially-lit environment. However,

Dracup et al. (1998c) noted that the responses in terms of flowering initiation and physiological maturity were considerably smaller with artificially extended days than that with naturally longer days, probably due to the higher threshold and saturation levels of illuminance for photoperiodic responses in lupin. Thus, the responses from these species to photoperiod under natural photoperiodic conditions may be different.

4.2 Pollination and pollen dispersal

Pollination habit differs among different lupin species, from self-pollinated, self-pollinated with facultative cross-pollination, to mandatory cross-pollinated. As discussed in Section 4.1, annual lupins are predominantly self-pollinated and perennial lupins are generally cross-pollinated. For annual lupins, there is also variation in outcrossing rates within a species for different genotypes, location and year of planting, with a close association with bee activity (Forbes et al. 1971).

Fertilization in self-pollinated species occurs in closed flowers and in the earliest phases of their development. Species in this category include *L. angustifolius* (Kazimierska & Kazimierski 2002) and *L. albus* (Williams 1991). However, no species has been found to be strictly self-pollinated. For example, in the case of *L. angustifolius*, the outcrossing rate has been shown to be low but may vary depending on a number of factors (see Section 9.1 for more detail). For *L. albus*, although pollination also occurs in very early phases of flower development, it has an outcrossing rate around 10% (Luckett 2010). If bitter and sweet albus varieties are grown near each other there is a high likelihood that pollen will be transferred between the varieties mainly through foraging honey bees. In sweet varieties, the introduction of the bitter gene via cross pollination poses a serious threat because, once introduced, the bitter gene frequency will increase with each generation and the overall alkaloid level of the bulk crop could exceed the allowable level (200 mg/kg) (Luckett 2010).

Lupin pollen is sticky and not suited to wind distribution (Hamblin et al. 2005; Langridge & Goodman 1977; Langridge & Goodman 1985). Therefore, cross-pollination among lupin plants mainly happens with the aid of bees and other insects. Insect pollinators not only act as agents of cross-pollination, but also have the function of inducing self-pollination (Pazy 1984). Kazimierska and Kazimierski (2002) state that lupin flowers do not produce nectar but lupin is still an entomophilous plant attracting insects by coloured flowers, nutritious pollen and a fragrance liquid from the vexillum. However, this is contradicted by the fact that beekeepers in Australia have used honeybee to collect nectar from cultivated *L. angustifolius* and albus lupin (Langridge & Goodman 1977; Langridge & Goodman 1985).

In Australia, the main lupin pollinator is the exotic honey bee (*Apis mellifera*). For example, honey bees represented 83% of the pollinators in WA (Manning 1995). Other lupin pollinators also include native bees (*Exoneura bicolor*, *Leioproctus* sp. and *Lasioglossum* sp.), and exotic bumblebee (*Bombus terrestris*) (Stout et al. 2002).

Information on the longevity of lupin pollen under natural condition is scarce. The pollen of *L. luteus* has been studied under some controlled conditions. In one case, the pollen of *L. luteus* was shown viable for pollination after 30 days of storage at 10°C (Kazimierska & Kazimierski 2002). In another study (Campos-Andrada 1999), *L. luteus* pollen viability assessed by *in vitro* pollen tube germination, was 10.8% and 1.5% after two years of dry storage at 3°C or -18°C and room temperature, respectively. However, pollen germination is

affected by temperature and humidity; temperatures below 12°C and above 36°C have a negative effect on the pollen germination process (Kazimierska & Kazimierski 2002).

4.3 Seed development and seed dispersal

4.3.1 Seed development

Lupin seeds develop within pods borne on terminal racemes of the main stem and branches. Flowering and pod setting occur on the main raceme first and then on the first, second and subsequent orders of branching. Dracup and Kirby (1996b) conducted a detailed study on pod and seed development of *L. angustifolius*; the process can be briefly described as follows:

In the immediate post-fertilisation phase, the seeds occupy most of the space between the pod walls, and then septa begin to form between seeds enclosing them in separate chambers. As the pod approaches maximum dry weight, the seeds fill proportionally more space until the pod walls are pushed apart and the septa broken. At physiological maturity, the seeds touch each other, and the volume of the seeds diminishes rapidly due to loss of water. As a seed develops, the embryo expands while the endosperm is progressively depleted and the embryo eventually occupies the whole space inside the seed coat. At this stage, the first and second pairs of leaves are visible, enclosed between the cotyledons. Although pods are set first on the main shoot, followed by the first and then the second-order branching, they reach maximum dry weight almost simultaneously on all branches.

The number of pods per plant and number of seeds in each pod varies among species. Additionally, the number of seeds per pod varies on the same plant. For annual lupin species such as *L. angustifolius*, each plant can bear around 30 – 40 pods and each pod contains 3 to 7 seeds, so that each plant can produce around 90 to 120 seeds (Clements et al. 2005b; Farrington & Gladstones 1974). However, perennial lupin species produce more pods and seeds. For example, *L. polyphyllus* can produce more than 1000 seeds per plant each year (Aniszewski et al. 2001).

Pod and seed set can be influenced by growing conditions including extreme temperature, drought and deficiency in certain nutrients. Temperature conditions before flowering have been shown to have a major influence on dry matter accumulation in inflorescences and on seed yield during the first 24 days after flowering (DAF), and temperature conditions after flowering also have an important effect on ultimate seed yield (Downes & Gladstones 1984). Furthermore, temperature during seed maturation can even affect embryo development and therefore affect subsequent crop performance as shown in *L. albus* (Clapham et al. 2000). Moisture stress at flowering and during seed filling has also been shown to have adverse effects on seed yield (Biddiscombe 1975). Lupin generally does not respond well to fertiliser nutrient, and trace element deficiencies, such as boron, can result in reduced pod set (Wong 2003).

4.3.2 Seed dispersal

Like other plant species in the legume family, lupin seed is dense without appendages and therefore is unlikely to be dispersed by wind over long distance. Long distance dispersal of lupin seeds can happen through waterways, animals and human activities.

Generally the movement of forage legume seeds can be achieved by adhesion to the coat of animals, ingestion and subsequent excretion in the faeces of herbivores such as sheep and

cattle, and non-herbivorous predators such as ants (Sulas et al. 2000). Lupin seeds do not have structures allowing attachment to animal fur or feather for long distance dispersal. According to Thomson et al. (1990), seeds heavier than 2 mg are unlikely to survive in large numbers after ingestion by sheep. The seed weight of common lupin species are more than 20 mg (Information portal for lupins 2010a), which makes lupin seeds less likely to survive after ingestion. However, one feeding study showed that *L. arboreus* seed can survive ingestion by deer at a low rate (Robinson 2010). Outside cultivation, lupin spread has been through waterways, by people dispersing seeds along roadsides and by roadwork contractors using gravel containing seeds. For instance, *L. polyphyllus* seeds are spread through transport by vehicles, soil transportation and other human activity (Fremstad 2006).

Without other dispersal vectors, the seeds of wild or naturalised lupin are dispersed mainly through mechanical dispersal (or ballistic dispersal) mode. When the seed pod becomes dry and brittle, the built-up torsion rips the pod apart and shoots seeds away from the parent plant, allowing the population to spread a couple of meters each year. For example, Nootka lupin (*L. nootkatensis*) seeds are commonly dispersed 1-3 metres from the mother plant and may expand by 1-2 metres annually on level ground (Magnusson 2006).

Modern lupin cultivars commonly carry genes, such as *lentus (le)* and *tardus (ta)*, for non-shattering or reduced-shattering pods (Boersma et al. 2007b; Cowling et al. 1998). Pods produced from cultivars carrying such genes are generally not shattered at maturity under normal condition, but warm and dry weather could increase seed shattering (Gladstones 1967). The main means of seed dispersal are then through human activities, such as soil movement and planting for agriculture (Spooner 2007).

4.4 Seed dormancy and germination

Impermeability of seed-coat to water and gases, or hardseededness, which leads to physical seed dormancy, is widespread in the Fabaceae family. This type of seed is commonly termed hard seed or orthodox seed, i.e. seed that can be stored in a state of low moisture for long time (Roberts 1973). The production of hard seeds with testa (seed coats) that are impermeable to water preventing germination of all seeds in any one year, is one of the survival adaptations of many plant species including wild lupins. Lupin displays the classical developmental pattern of orthodox seeds (Garnczarska et al. 2009). Lupin seed dormancy is a physical process that appears to be induced after maturity as the seed moisture content reduces to a certain level (Boersma et al. 2007a). According to the study on the Western Australian blue lupin (*L. digitatus* Forsk.) by Gladstones (1958), seeds remain fully permeable when moisture content is above 14% and permeability declines when moisture drops below 14%. All seeds become impermeable when moisture decreases to 11% and practically irreversible status is reached when moisture is below 9%.

Lupin seed dormancy varies widely among and within species due to genetic and environmental factors. Wild lupins are generally hard-seeded and can remain dormant for long periods unless softened by change of environmental conditions. For example, *L. arcticus* seeds buried in a Canadian peat bog in permanently frozen silt for an estimated 10,000 years were able to germinate and produce healthy, flowering plants (Porsild et al. 1967). Seed bank size and seedling recruitment of wild lupin are influenced mainly by three factors that affect seed demography: (1) post-dispersal seed predation by granivores, such as rodent, (2) seed viability, and (3) seed dormancy (Maron & Simms 1997). Daily temperature fluctuations have a positive effect on softening hard seeds (Arrieta et al. 1994; Quinlivan 1966). In the case of

sand-plain lupin (*L. varius*), daily temperature fluctuations between 15 and 65°C can effectively fracture the impermeable coat at the strophiole⁴ of the hard seeds to make them permeable for germination (Quinlivan 1968).

Modern lupin cultivars are bred to be soft-seeded (permeable to water and gases), which allows uniform germination for stable seed production. Seeds of commercial lupin cultivars have high permeability to water due to the presence of the gene *mollis* for soft seed (Gladstones 1977; Mikolajczyk 1966). Seed dormancy is short and seed harvested at physiological maturity can germinate immediately (Perry et al. 1998). Viability of such seeds is influenced by moisture content in the seeds, and temperature and relative humidity in the storage environment (Thomas et al. 2008b). Both viability and seedling vigour decrease if seeds are maintained in dry soil only partially imbibed (Dracup et al. 1993).

Desiccation is not required for the onset of ability to germinate in lupin. Garnczarska et al. (2009) showed that the ability of freshly harvested *L. luteus* seeds to germinate began at about 25 DAF, which was before reaching physiological maturity at around 40 DAF. However, such immature seeds tend to produce abnormal seedlings. Only seeds harvested after physiological maturity have the full potential to survive desiccation and subsequently germinate after rehydration.

Water availability and temperature are the most important factors for germination and emergence of lupin. Lupin is generally tolerant to cold and drought. For *L. angustifolius*, the base temperature for germination was 0-3°C at a normal soil matric potential⁵ of -0.003 megapascal (MPa) (Dracup et al. 1993). Germination rate increased linearly with temperature up to 20°C and then decreased at higher temperature; at less than 22°C, germination was close to 100% but declined to 27% at 30°C. Therefore, the optimum temperatures for lupin germination and growth are close to 20°C. On the other hand, when soil temperature was maintained at 15°C, germination rate declined with decreasing soil matric potential from -0.003 MPa to the germination threshold at -2 MPa. No germination was observed at the soil matric potential of -2.2 MPa.

4.5 Vegetative growth

Other pulses such as chickpea, faba bean, lentil and field pea, have hypogeal emergence and their cotyledons remain where the seed is sown and only the shoot emerges from the soil surface. In contrast, lupin species have epigeal germination pushing both cotyledons above the soil surface. Subsequently, sowing lupins below 4 cm reduces crop emergence and establishment (Siddique et al. 1997). Early seedling growth is slower than later vegetative stages and maximum vegetative growth rate occurs during flowering.

Most lupins have a dominant tap-root. Mature plants can have a rooting depth exceeding 2 meters on favourable soil types, and proteoid roots can develop in response to deficiency of certain nutrients in soil (see Section 3.1). After inoculation with bradyrhizobia, nodulation will occur on lupin roots for nitrogen fixation.

⁴ A strophiole is an outgrowth or tubercle around the hilum of certain seeds.

⁵ Soil matric potential is the force placed on water by the soil matrix. In water saturated soil it is near zero. As the soil dries, matric potential becomes more negative and it takes more energy for plant to extract water from the soil. It is often referred to as soil water “tension”.

L. angustifolius has an indeterminate growth habit composed of determinate terminal inflorescences on the main stem and lateral branches. During the vegetative stage, the growth of the main stem is slow and determined by the elongation of the internodes. At flowering, the main stem accounts for only 10-15% of the total biomass at physiological maturity, which is due to the determinate growth imposed by the terminal inflorescence. After main stem flowering, the plant still invests in growth of apical branches. However, there is a limit to the number of branch orders produced even under well watered and high fertility conditions. Apical branch growth ceases after the emergence of the fifth order of branches (Palta et al. 2008). More detailed description of *L. angustifolius* growth can be found in the Lupin Development Guide (Dracup & Kirby 1996a).

Duration of the vegetative stage varies depending on lupin varieties, as well as year and place of cultivation. In Russia, Belarus and the Ukraine, duration of vegetative growth in lupins sown in winter is from 72 to 170 days for *L. angustifolius*, from 90 to 175 days for *L. luteus*, and from 106 to 180 days for *L. albus* (Kurlovich & Kartuzova 2002). In Australia, winter sown lupins have about 75 to 100 days of vegetative growth (Perry et al. 1998).

SECTION 5 BIOCHEMISTRY

5.1 Nutrient components of the lupin seed

Lupin, like other grain legumes, is a source of high-quality protein, essential amino acids, oil and other nutritive substances. The major biochemical feature of lupin is the capability to synthesize a high proportion of protein. Due to its coexistence with nodule bacteria, lupin possesses high nitrogen-fixing ability to acquire nitrogen from the atmosphere for producing protein and other nitrogen substances (Kurlovich et al. 2002a). Proximate analysis for whole seeds and kernels of the major lupin crop species are shown in Table 4.

Table 4. Nutrient composition of seeds and kernels of the major lupin species *

Component	<i>L. angustifolius</i>		<i>L. albus</i>		<i>L. luteus</i>		<i>L. mutabilis</i>	
	whole seed (%)	kernel (%)	whole seed (%)	kernel (%)	whole seed (%)	kernel (%)	whole seed (%)	kernel (%)
Moisture	9	12	9	11	9	12	8	10
Protein	32	41	36	44	38	52	44	52
Fat	6	7	9	11	5	7	14	17
Ash	3	3	3	4	3	4	3	4
Crude fibre	15	9	10	2	13	2	7	10
Lianin	<1	<1	<1	<1	<1	<1	<1	<1
NSP	22	29	17	21	8	11	9	10
Oligosaccharides	4	6	7	8	9	12	5	6
Starch	ND	-	ND	-	ND	-	-	-

*Sources: (Information portal for lupins 2010c; Petterson 1998). NSP, non-starch polysaccharides; ND, not detectable.

In contrast to crops such as field peas and chickpeas, which have 50-70% of the cotyledon weight as starch, there is very low amount of starch in the seeds of any lupin crop species (Pettersen 1998). Therefore, all lupin food ingredients have close to zero Glycemic Index (GI)⁶ (Information portal for lupins 2010c).

5.1.1 Proteins and amino acids

Lupin seed storage protein is made up of a large proportion (85%) of globulins and a small proportion (15%) of albumins (Pettersen 1998). The globulins and albumins are also referred to as conglutins (α , β , γ and δ conglutins) (Blagrove & Gillespie 1975; Foley et al. 2011), some of which may act as allergens (see more details in Section 5.2).

The globulin fraction contains three major proteins: α -, β - and γ -conglutins. α -conglutin belongs to the family of 11S or 'legumin-like' globulins consisting of hexamers of two disulphide-linked heterogeneous subunits, one being an acidic subunit (31, 36, 42 or 46 kDa) and the other being a basic subunit of 19 kDa. β -conglutin belongs to the family of 7S or 'vicilin-like' globulins. It has a trimeric structure consisting of up to 10 to 12 polypeptides (with molecular weight range from 15 to 72 kDa) with no disulphide bridges (Melo et al. 1994).

γ -conglutin is a lupin specific globulin (Salmanowicz 1995). It is a basic, monoglycosylated tetrameric 7S protein consisting of two subunits (17 and 30 kDa) linked by disulphide bonds (Duranti et al. 1981; Restani et al. 1981). This protein has recently attracted more attention due to its unique glucose-controlling properties (Magni et al. 2004).

Lupin albumins are acidic proteins soluble at pH 5 and vary in size from 6 to 117 kDa. The 2S proteins (including δ -conglutin) constitute the major component of the seed albumin fraction (Salmanowicz 1995). δ -conglutin is a 2S protein, which was referred to the 2S albumin class due to high degree of homology between their primary structures (Salmanowicz 1995). It consists of two polypeptide chains (4.6 and 9.4 kDa) linked by two disulphide bonds (Duranti et al. 1981).

Compared to other grain legumes such as peas, soybean and string bean, lupins appear to contain the least amount of proteins having anti-nutritious properties: inhibitors of proteinase and hemagglutinins (lectins). They are practically absent in the main cultivated species and cultivars (Kurlovich et al. 2002a).

The amino acid profile of lupin seed proteins is high in arginine, lysine, leucine and phenylalanine when compared to soybean. The notable difference is the comparative deficiency of methionine and cysteine (Glencross 2001).

5.1.2 Carbohydrates

Lupins are typically low in starch and most species contain less than 1.5% in the seeds. Therefore, the non-starch polysaccharides (NSP) constitute the major portion of the carbohydrate fraction of all lupin species, typically being about 40% (Glencross 2001). Lupin

⁶ GI is a measure of the effects of carbohydrates on blood sugar levels (Jenkins et al. 1981). Carbohydrates that break down more slowly, releasing glucose more gradually into the bloodstream, have a low GI.

seed hull and cotyledon contain different types of carbohydrates. The hull is predominately composed of structural NSP: cellulose, hemi-celluloses and pectins. In contrast, the main NSP in the cotyledons are the non-structural polysaccharides of the cell walls, with the main constituent sugars being galactose, arabinose and uronic acids (Pettersen 1998).

5.1.3 Lipids

As shown in Table 4, the lipid content varies considerably among different lupin species. The composition of total lipids, with the whole seed of *L. angustifolius* as example, is: triacylglycerols (or triglycerides, 71.1%), phospholipids (14.9%), free sterols (5.2%), glycolipids (3.5%), sterol and wax esters (0.5%), free alcohols (0.4%), hydrocarbons (0.4%) and unidentified waxy material (0.4%) (Glencross 2001). The main fatty acids present are: linoleic (48.3%), oleic (31.2%), palmitic (7.6%) and linolenic (5.4%) (Van Barneveld 1999).

5.2 Toxins

Most wild lupin species are considered to be toxic due to their high content of quinolizidine alkaloids (Keeler 1989). Lupins are also associated with a mycotoxicosis called lupinosis caused by phomopsins (Allen 1998). However, toxins in commercially grown lupins have generally been reduced to manageable levels as a result of domestication and breeding (Cowling et al. 1998).

5.2.1 Alkaloids

There are many toxic alkaloids present in the genus *Lupinus*, including pyrrolizidine and piperidine alkaloids (Panter et al. 1998). The lupin alkaloids in the species of agricultural importance are usually derivatives of quinolizidine and, therefore, called quinolizidine alkaloids (QAs). Over 100 QAs have been reported in the genus *Lupinus* (Wink et al. 1995). The main role of the alkaloids is to provide the plant a chemical defence against herbivores (Wink 1992). Some of the alkaloids may also display antimicrobial activities and confer resistance to bacterial and fungal pathogens (Erdemoglu et al. 2007; Wink 1988). However, alkaloids make lupin seeds bitter and are toxic when ingested by human or animals.

QAs are mainly in the forms of bicyclic (eg lupinine), tricyclic (eg angustifoline) and tetracyclic (eg lupanine, 13-hydroxylupaine, sparteine, multiflorine and α -isolupanine) (Pettersen 1998). Different lupin species have different profiles of alkaloids, and within a species there are usually four or five major alkaloids and several minor ones (Allen 1998). Wild lupins have more complex alkaloid profiles than the domesticated lupin cultivars. For example, *L. hintonii*, a wild lupin species grown in the central highland of Mexico, contains at least 19 QAs with six major ones in leaves and seeds (Torres et al. 2002). The QA profiles for seeds of some important lupin species are shown in Table 5. However, total alkaloid concentrations range from 0.01 to 4%, depending on the species, plant part and growing conditions (Allen 1998).

Table 5. Quinolizidine alkaloid composition (percentage of total alkaloids) in seeds of the major lupin species*

Alkaloid	<i>L. angustifolius</i>	<i>L. albus</i>	<i>L. luteus</i>	<i>L. mutabilis</i>
Albine	-	15	-	-
Ammodendrine	-	-	-	2
13-angeloyloxylupanine	-	-	-	2
Angustifoline	10	-	-	1
3-hydroxylupanine	-	-	-	12
13- hydroxylupanine	12	8	-	12
Lupanine	70	70	-	46
Lupinine	-	-	60	-
Multiflorine	-	3	30	-
Sparteine	-	-	-	16
Tetrahyrorrhombifoline	-	-	-	2

*Source: (Petterson 1998; Wink et al. 1995). '-', not detected in chromatogram.

The distribution of alkaloids in plant organs is uneven; some plants accumulate them mostly in seeds and others in vegetative tissues such as leaves, roots and cortex. Alkaloid content undergoes changes throughout the lupin growth period with the peak at the flowering phase (Maknickiene & Asakaviciute 2008). Toward the end of the life cycle, alkaloids accumulate in seeds and roots (Hondelmann 1984).

Lupin alkaloids can impact the central nervous systems of mammals, with low levels acting as stimulators and higher levels as suppressors (Maknickiene & Asakaviciute 2008). QA intoxication is characterized by trembling, shaking, excitation, and convulsion, and can lead to anticholinergic syndrome with blurred vision, dry mouth, nervousness and malaise (Kurzbaum et al. 2008). Lupanine and sparteine are the most common QAs that show acute oral toxicity due to neurological effects leading to the loss of motor co-ordination and muscular control (Resta et al. 2008a). The food safety and health authorities of some countries, including France, UK, Australia and New Zealand, have set the maximum limit of alkaloid content in lupin flours and food at 200 mg/kg (Resta et al. 2008b).

5.2.2 Phomopsins

Phomopsins are toxins produced by the fungus *Diaporthe toxica* (formerly known as *Phomopsis leptostromiformis*, and less frequently referred to as *Diaporthe woodii* and *Phomopsis rossiana*), which colonises lupin plants, and can lead to a mycotoxicosis called lupinosis if ingested by grazing animals (Allen 1998; Petterson 1998). Lupinosis is primarily a disease of sheep, but has also been reported in cattle, goats, donkeys, horses and pigs (Allen 2009). The disease has also been induced experimentally in rabbits, guinea pigs, mice, rats, dogs, ducklings and chickens (Allen 2009). No direct evidence of phomopsin toxicity in humans is available due to the paucity of exposure data (ANZFA 2001). The classical clinical signs of lupinosis are lack of appetite, loss of condition, lethargy, jaundice and often death as a result of severe liver damage (Allen 1998).

The phomopsins are a group of low molecular weight macrocyclic hexapeptides (ANZFA 2001; Culvenor et al. 1989). Five phomopsins have been identified and they are named phomopsins A, B, C, D and E (Allen 1998). Phomopsins A and B were the first two isolated and shown to be capable of inducing lupinosis in sheep and young rats (Culvenor et al. 1977).

Phomopsin A makes up about 80% of the toxic extracts and is therefore considered to be the principal toxin responsible for lupinosis. Phomopsin B is the des-chloro analogue of phomopsin A (Allen & Hancock 1989). Phomopsins C, D and E attract less attention due to the very low-level presence in lupin plants, but all of these components are capable of producing mitotic disturbances in the hepatocytes of nursing rats similar to those produced by phomopsin A (Allen 1998).

D. toxica grows mainly within lupin stems and causes *Phomopsis* stem-blight, which produces sunken, linear stem lesions but it also affects leaves, pods and seeds. The fungus is also a saprophyte and grows well on dead lupin materials such as haulm (stalks or stems), pods and stubble. Infection of the live plant is latent and signs of the infection do not normally show until the plant matures and senesces (Williamson et al. 1991; Wood & Brown 1975). Phomopsin production in quantities sufficient to cause animal disease is associated with the presence of visible lesions on the lupin stems, pods or seeds. Lupinosis is therefore a disease of summer and autumn, when the dead plants are eaten (Allen 1998).

5.3 Allergens

A small percentage of people have food sensitivity to lupin; a recent study reported at least 151 cases of lupin allergy from some EU countries, the USA and Australia through a literature search of published data (Jappe & Vieths 2010). In Australia, there have been a few reported reactions to lupins, affecting ten adults (Campbell et al. 2007; Smith et al. 2004). Allergic reactions to lupin seed, flour or dust have been reported but evidence for sensitisation by pollen inhalation is particularly poor (Jappe & Vieths 2010). The most common clinical conditions reported are anaphylaxis, urticaria, asthma, conjunctivitis, oedema and oral allergy syndrome (Sanz et al. 2010). Occupational sensitisation to lupin with asthma, rhinitis and conjunctivitis has also been studied, and shows a sensitisation rate as high as 29% (Campbell & Yates 2010). However, there has been no report of death associated with hypersensitivity to lupin.

The major allergens of the *Lupinus* species are storage proteins, the conglutins, as described in Section 5.1 (Jappe & Vieths 2010). The β -conglutin from *L. angustifolius* has been designated as the allergen Lup an 1 by the International Union of Immunological Societies Allergen Nomenclature Subcommittee (Goggin et al. 2008).

In addition to this formally recognized lupin allergen, reactivity of lupin conglutins to lupin-specific immunoglobulin E (IgE) and cross reactivity of lupin conglutins with other legumes, particularly peanut, have been studied (Jappe & Vieths 2010; Sanz et al. 2010). γ -conglutin and the basic subunit of α -conglutin from *L. albus* have been shown to be IgE-reactive with sera from lupin allergic patients (Magni et al. 2005). Cross reactivity between the 2S albumin-related δ -conglutin of *L. angustifolius* and the peanut protein allergen Ara h 2 has also been reported (Dooper et al. 2009).

5.4 Other undesirable phytochemicals

Oligosaccharides, phytic acid and glycosides (such as saponins) are some of the undesirable compounds found in lupins (Allen 1998). Although traditionally they have been known as anti-nutritional factors, these compounds may also have beneficial effects, such as antioxidant effects and prevention of cancer (Pettersen 1998; Rochfort & Panozzo 2007).

5.4.1 Soluble polysaccharides and oligosaccharides

The water-soluble portion of the carbohydrates of the lupin seeds (about 5%) is considered to have an anti-nutritional effect due to its viscous nature and effects on intestinal transit time and changes in hormonal regulation due to differential nutrient absorption rates.

The lupin oligosaccharides belong to the raffinose family, which are α -galactosyl derivatives of sucrose that cannot be metabolised by monogastrics. When they pass through to the colon, bacterial digestion breaks them down to produce carbon dioxide, methane and hydrogen, which can cause abdominal discomfort and cramps and result in flatulence (Petterson 1998).

5.4.2 Phytic acid

Phytic acid may reduce the bioavailability of minerals in monogastric animal diet through chelation of mineral cations, such as zinc, copper, cobalt, calcium, iron, potassium and magnesium, to form nonabsorbable phytates. The phytic acid content in cultivated lupins is below 1%, which is less than in barley, wheat and soybean (Allen 1998).

5.4.3 Saponins

Saponins are glycosides present in many plants with a bitter taste. Both adverse and beneficial effects of these compounds to animals have been reported (Francis et al. 2002). Their adverse effects are mainly reflected in depressed feed intake that causes growth inhibition to animals, monogastrics in particular, and reduced animal reproduction. Their anti-nutritional effects may be related to an increase of the permeability of the small intestinal mucosa cells, which leads to an inhibition of active nutrient transport (Johnson et al. 1986). The negative effects of saponins on animal reproduction have long been known to be associated with their abortifacient, anti-zygotic and anti-implantation properties (Francis et al. 2002).

Saponin content varies among different lupin species. *L. albus* contains a negligible level (Petterson 1998) and *L. luteus* has a moderate amount at 55 mg/kg (Cuadrado et al. 1995). However, the level in *L. angustifolius* is higher at 480 to 730 mg/kg (Ruiz et al. 1995). This is still much less than that in soybean and field pea, which are 3500 and 1800 mg/kg, respectively (Allen 1998).

5.5 Beneficial phytochemicals

As mentioned in Section 5.4, some of the phytochemicals that were traditionally considered anti-nutritional factors may also have beneficial effects. Oligosaccharides have potential value for immune health. Both saponins and phytic acid are involved in anticancer and hypocholesterolemic action. For more details and references, please refer to a review by Rochfort and Panozzo (Rochfort & Panozzo 2007).

Lupin is a rich source of dietary fibre, which includes polysaccharides, oligosaccharides and lignin (Pisarikova & Zraly 2010). According to Tucek (2009), lupin produces two distinct types of dietary fibre: (a) the thick seed coat (hull or bran) and (b) kernel fibre. The seed coat comprises 10 to 30% of the seed weight. This percentage varies according to the species but 25% is a typical amount. The kernel fibre comprises the cell wall component of the lupin seed kernel and accounts for about 30 to 40% of the kernel weight. There are significant physical and chemical differences between lupin hull and kernel fibre, relating to colour, chemical

composition (see also Carbohydrates under Section 5.1.2), functional characteristics and nutritional value.

Small quantities of lupin bran (about 200 tonnes per annum) are used in Australia in a limited range of bread products for dietary fibre fortification (Tucek 2009). Lupin kernel fibre consists primarily of insoluble cell wall material and has a chemical structure similar to pectin (Evans et al. 1993), which is a soluble fibre known to reduce cholesterol levels. A high-fibre diet incorporating lupin kernel fibre showed favourable changes to some serum lipid measures in healthy men, suggesting that this fibre may be useful in the dietary reduction of coronary heart disease risk (Hall et al. 2005).

SECTION 6 ABIOTIC INTERACTIONS

Lupins are known for their ability to thrive on soils of low fertility. However, there is distinct variation among lupin species in responses to various abiotic stresses, given the range of environments in which lupins are distributed. Lupins characteristically grow on well-drained acidic to neutral soils and are generally intolerant to extremely alkaline or saline soils and waterlogging (Dracup et al. 1998b).

6.1 Abiotic stresses

6.1.1 Nutrient stress

Due to their capability to fix atmospheric nitrogen through nodulation of nitrogen fixation bacteria, most lupin crops are used as a source of nitrogen in farming systems and nitrogen deficiency is generally not a concern. However, deficiency of other minerals such as phosphorus, cobalt, copper and iron can all affect nodulation and nitrogen fixation and therefore lead to nitrogen deficiency (Longnecker et al. 1998). For some nutrients, such as phosphorus, manganese and boron, stress comes from either deficiency of the element, which leads to reduced growth or other abnormal symptoms, or toxic effects when the concentration of the element exceeds certain levels in the plant (Brennan et al. 2008).

Phosphorus

Without water limitation, inadequate phosphorus is frequently the limiting factor for lupin growth. Seed phosphorus concentration has been shown to have an effect on early vigour and even final grain yield (Bolland et al. 1990). Seedlings of *L. angustifolius* grown from seed with low phosphorus concentration (less than 0.021 %) had decreased early growth even if adequate phosphorus was supplied (Thomson et al. 1992). Phosphorus deficiency in soil also limits vegetative growth and nodulation, and leads to decreased harvest index for *L. angustifolius* (Jarvis & Bolland 1991).

Iron

Iron deficiency is one cause of the poor growth of lupin on fine-textured alkaline soils (White & Robson 1989b). The primary symptom of iron deficiency is interveinal chlorosis, the development of a yellow leaf with a network of dark green veins. When grown on the same alkaline soil deficient in iron, *L. angustifolius*, *L. luteus* and *L. albus* showed more severe chlorosis than *L. atlanticus*, *L. pilosus* and *L. cosentinii* (Tang et al. 1995).

Zinc

In general, lupin crops are less susceptible to zinc deficiency than cereal crops such as corn, wheat and oats. Sensitivity to zinc-deficient soil also varies among species. *L. albus* is more sensitive than *L. angustifolius* and sensitivity to zinc deficiency has not been observed in rough-seeded *L. atlanticus* and *L. pilosus* (Longnecker et al. 1998). On the other hand, excessive zinc can cause phytotoxicity. Pastor et al. (2003) showed that *L. albus* growth was severely affected when zinc concentration in soil exceeded 300 ppm.

Cobalt

Cobalt is not required by lupins but it is required by the Bradyrhizobia (nitrogen fixing bacteria) in root nodules (Brennan et al. 2008). Cobalt deficiency is likely to limit nitrogen fixation by effects on both multiplication of bradyrhizobial and nodule function. *L. angustifolius* is particularly sensitive to cobalt deficiency. Seeds with low cobalt concentrations sown into soils deficient in cobalt will produce poorly-nodulated roots with ineffective nodules (Brennan et al. 2008).

Manganese

Grain yields of lupin can be substantially reduced by manganese deficiency, but shoot yields are generally not affected (Brennan 1999). *L. angustifolius* has a poor ability to accumulate manganese in its grain and low availability is a common problem in soils used for production of *L. angustifolius* in WA (Longnecker et al. 1998). Manganese deficiency leads to split seed disorder (also called split seed syndrome) and sometimes to discolouration around the margins of the split seed (Brennan et al. 2008; Perry & Gartrell 1976; Walton 1978). The seed may also be small, shrivelled and poorly developed. Plants suffering from this deficiency show delayed maturity and produce lower yields (Brennan et al. 2008). The viability of seeds with manganese content less than 13 mg/kg, is greatly reduced compared with concentrations higher than 13 mg/kg (Brennan & Longnecker 2001).

Boron

There is a narrow range of boron levels in soil between deficiency and toxicity for most crop species (Brennan et al. 2008). Lupin plants grown in soils deficient in boron may have reduced pod set (Wong 2003).

6.1.2 Temperature stress

As mentioned in Section 4.4, the optimum temperature for lupin germination and growth is around 20°C.

Low temperature

Lupins are generally cold tolerant. For a majority of lupin species, minimum temperature for seed germination is low at about 2 to 3°C (Kurlovich & Heinanen 2002). According to Barbacki (1960), annual lupin species are capable of enduring severe frost. For instance, *L. albus*, *L. luteus* and *L. angustifolius* can tolerate temperatures as low as -6, -8 and -9°C, respectively. However, tolerance to low temperatures also depends on the interaction of a genotype with ecological and geographical features and the phase of plant development.

High temperature

Germination and emergence of *L. angustifolius* are reduced when soil temperature is higher than 20°C, with almost no germination and emergence at 30°C (Dracup et al. 1993).

Reproductive tissues are particularly sensitive to high temperature. Likely consequences of high temperatures (above 30°C) around flowering include male sterility, reduced pollen tube elongation, and lowered pod and seed set (Dracup et al. 1998b).

6.1.3 Water stress

Water deficit

In the Mediterranean environment of WA, lupin yields have been variable, largely attributed to the amount and distribution of rainfall, and the water-holding capacity of the soil. For example, drought terminates the growing season of *L. angustifolius*, and the timing and intensity of this terminal drought are among the main causes of the variability of yield and harvest index (Dracup et al. 1998a). Water deficit during seed filling can hasten seed development and cause pod and seed abortion, therefore the effect of terminal stress is greater on the later formed pods (Dracup & Kirby 1996b).

Under water-stress conditions, lupin switches quickly from vegetative to reproductive mode, shortening the post-flowering phases, and the duration of pod and seed-filling (Dracup & Kirby 1996b; French & Turner 1991). However, lupins are also able to avoid reproductive failure caused by water deficit through accumulating reserves (eg sugars) in certain organs (Pinheiro et al. 2001; Rodrigues et al. 1995). Rodrigues et al. (1995) showed that *L. albus* responded to water deficit during flowering by losing 50% of the total leaf canopy and increasing stem dry weight by 55%, whilst maintaining total seed production.

Waterlogging

Lupins are considered relatively intolerant to waterlogging, although tolerance within the genus varies. The adaptation of some cultivated lupin species to waterlogging is shown in Table 2 in Section 2.3.2. Symptoms of waterlogging include wilting, chlorosis and pigmentation of the oldest leaves and cotyledons, but which of these responses occur first depends on the species and conditions (Davies et al. 2000; Dracup et al. 1998b).

Seeds, seedlings and mature plants respond to waterlogging differently. The growth stage at which waterlogging occurs and its duration are important in determining the overall damages. Lupin seeds are sensitive to waterlogging. For example, in waterlogged soil *L. angustifolius* seeds did not germinate and died within four days (Sarlistyaningsih et al. 1995). Generally, waterlogging decreases growth of roots and root extension is particularly sensitive (Jackson & Drew 1984). Secondary to those on roots are the effects of waterlogging on shoots and stem elongation, leaf expansion and dry matter accumulation. During waterlogging, yellowing of the cotyledons and chlorosis of the older leaves occur, and the rate of growing leaf expansion reduces (Davies et al. 2000).

Waterlogging also limits symbiotic nitrogen fixation by bradyrhizobia (Dracup et al. 1998b). When the external oxygen concentration declines (as a result of waterlogging for example), acetylene reduction by lupin nodules also declines (Trinick et al. 1976). Waterlogging may

lead to the breakdown of the nodules, but once it is relieved, the plant is able to form new nodules to fix nitrogen (Farrington et al. 1977).

6.1.4 Other stresses

Salinity

Lupins do not tolerate high levels of salinity (Dracup et al. 1998b). Lupin species vary in their tolerance but generally are moderately sensitive to salinity. Symptoms of salt toxicity in lupin include a gray blotching of leaflets first, then developing marginal to complete leaf necrosis followed by wilting and abscission of leaflets (Munns et al. 1988; Treeby & van Steveninck 1988).

6.2 Abiotic tolerances

Lupins are tolerant to a range of heavy metals. Those tested include: aluminium (Penaloza et al. 2000), arsenic (Vazquez et al. 2006), cadmium (Carpena et al. 2003; Page et al. 2006; Vazquez & Carpena-Ruiz 2005; Ximénez-Embún et al. 2001; Zornoza et al. 2002), chromium and lead (Gwozdz et al. 1997; Page et al. 2006; Ximénez-Embún et al. 2001), nickel (Page et al. 2006) and mercury (Page et al. 2006; Zornoza et al. 2010). In Europe, lupin plants have been tested and shown potential to be used for bioremediation based on their ability to solubilize and absorb elements through extensive cluster roots, with the help of nodulation with *Bradyrhizobium* (Fernandez-Pascual et al. 2007).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Common weeds found in lupin crops in Australia are listed in Table 6. Among them, annual ryegrass and wild radish are the major weeds, both severely reduce lupin yield (Harries et al. 2008). They compete for space, light and nutrients with the lupin crop. According to Harris et al. (2008), for each radish plant/m² or 25 ryegrass plant/m², there is a 5 % reduction in lupin yield.

Table 6. Common weeds in lupin crops in Australia*

Common name	Scientific Name
Barley grass	<i>Hordeum leporinum</i>
Brome grass	<i>Bromus</i> spp.
Capeweed	<i>Arctotheca calendula</i>
Doublegee	<i>Emex australis</i>
Ryegrass	<i>Lolium rigidum</i>
Silver grass	<i>Vulpia</i> spp.
Wild mustard	<i>Sinapis arvensis</i>
Wild oat	<i>Avena fatua</i>
Wild radish	<i>Raphanus raphanistrum</i>
Wild turnip	<i>Brassica tournefortii</i>
Wireweed	<i>Polygonum aviculare</i>

*Source:(McClarty & Harries 2009; Perry et al. 1998)

A number of weed management systems have been applied to lupin crops, including crop rotations involving wheat:lupin and lupin:wheat:canola, crop topping or swathing to reduce seed set by weeds and using clean-up crops such as swathed barley or hay production.

Weed management of lupin crops relies heavily on the use of herbicides. Commonly used herbicides include (Harries et al. 2008; McLarty & Harries 2009):

- before sowing – 2,4-D, atrazine, diquat, diuron, glyphosate, and paraquat
- Pre-emergence – atrazine, diuron, pendimethalin, simazine, triallate and trifluralin
- Post-emergence – butroxydim, clethodim, diflufenican, fluazifop, haloxyfop, metosulam, metribuzin, picolinafen, quizalofop and simazine
- Pre-harvest – paraquat.

However, with the continuous development of resistance to commonly used herbicides, weeds in lupin crops, particularly ryegrass and wild radish, have become increasingly more difficult to control. The effectiveness of herbicides on weeds and the amount of damage they may cause to lupin crops depends on a wide range of factors such as crop and weed growth stage, soil type, location, lupin variety and weather conditions at the time of herbicide application (McLarty & Harries 2009).

7.2 Pests and diseases

7.2.1 Pests

Vertebrate pests

Reports on vertebrate pests of lupin are scarce. The house mouse (*Mus domesticus*) and the introduced feral pig (or wild boar) (*Sus scrofa*) are the two major animal pests affecting lupin production in Australia.

The house mouse can cause considerable losses to lupin crops by eating recently-sown or germinating seedlings, seed heads of maturing lupin and stored grain when the numbers are reasonably high (Vertebrate Pest Research Section Forresterfield 2003). For example, mouse damage to lupin crops has been observed in the Lachlan and Riverina, NSW for some time (Henderson 2011).

Feral pigs can affect lupin production mainly by trampling and destroying crops. In WA, individual losses to lupin crops may reach tens of thousands of dollars (Choquenot et al. 1996). In 2004, feral pigs were believed to have increased in numbers and distribution in WA partly due to increased crops of white lupins, which is a preferred pig food (Cowled et al. 2004).

Invertebrate pests

Lupin crops are more prone to invertebrate pest damage than cereal crops. The major pests affecting lupin crops include caterpillars, Lucerne fleas, mites, slugs, snails, aphids and thrips (see Appendix 3).

In the establishment phase, lupins are vulnerable to attacks by caterpillars (cutworms and brown pasture looper), lucerne flea, mite, fly, slug and snail. In severe cases of uncontrolled

pest outbreaks at the seedling stage, it may be necessary to re-sow paddocks (Berlandier 2003).

At flowering stage, lupins are frequently attacked by aphids and thrips. Both aphids and thrips have numerous generations throughout the year. Cowpea aphids, blue green aphids and green peach aphids are responsible for most of the aphid infestations of lupin crops (Mangano et al. 2008). Susceptibility to aphid feeding damage varies among lupin species or even among different genotypes within the same species. For example, *L. luteus* is generally more susceptible than *L. angustifolius* but different varieties of *L. angustifolius* vary from susceptible to resistant (Berlandier 1999). Aphids appear to thrive in dry weather conditions and crops grown in low rainfall zones (less than 325 mm) appear to be at greatest risk (Berlandier 1999). Thrips, mainly onion thrips and plague thrips, may cause flower abortion when in high numbers but they rarely cause damage sufficient to warrant control (Mangano et al. 2008).

At podding stage, the larvae of Australian native budworm and lucerne seed web moth feed on seeds within the pods. Only a single generation of native budworm develops on lupins (Sweetingham et al. 1998), whereas lucerne seed web moth has three to four generations each year (Mangano et al. 2008). Newly hatched native budworm caterpillars feed on foliage and only larger caterpillars (over 15 mm long) will feed on lupin pods. Lucerne seed web moth is a very sporadic pest of lupins, causing notable damage only every 8 to 10 years (Berlandier 2003).

7.2.2 Diseases

Lupins are susceptible to a wide range of diseases. Although lupin diseases caused by bacterial pathogens have been reported (Lu & Gross 2010), most lupin diseases of agricultural importance in Australia are caused by fungal or viral pathogens (Appendix 4). In Australia, lupins are grown predominately in regions with a Mediterranean climate, which favours pathogens that are well adapted to survive the hot dry summer (Sweetingham et al. 1998).

Fungal diseases

The common fungal diseases of lupins are listed in Appendix 4. In Australia, anthracnose caused by *Colletotrichum lupini* (formerly named *Colletotrichum gloeosporioides*), brown leaf spot and root rot by *Pleiochaeta setosa* and phomopsis by *Diaporthe toxica* are the most important lupin diseases.

Anthracnose is a serious disease of lupins worldwide. In Australia, all lupin species of agricultural importance are affected but susceptibility varies among different species and among different cultivars of the same species. Generally, *L. albus* and *L. luteus* are more susceptible than *L. angustifolius* (see Appendix 2). Anthracnose has been found in most lupin producing areas of WA and SA, but it is most serious in the high rainfall zone of the northern agricultural region in WA (Thomas 2003). The disease is not known to occur in lupin crops in NSW, Victoria or Tasmania (Davidson et al. 2007).

The fungal pathogen *P. setosa* is responsible for both brown leaf spot and *Pleiochaeta* root rot diseases of lupins. It has affected lupin production in all continents where lupins are cultivated and is the most widespread and damaging pathogen of lupins in Australia, particularly in WA (Sweetingham 1997; Sweetingham et al. 1998). Most cultivars of

L. angustifolius and *L. albus* are susceptible to *P. setosa* but some cultivars of *L. luteus* show high degree of resistance (Thomas et al. 2008a) (also see Appendix 2).

Phomopsis, which causes lupinosis in livestock, has been discussed in Section 5.2. Phomopsis can reduce crop yields when lesions develop on stressed lupin plants prior to maturity, which results in lodging of the plants (Thomas et al. 2008a).

Viral diseases

Although no lupin-specific virus has been reported, cucumber mosaic virus (CMV) and some potyviruses, including Bean yellow mosaic virus (BYMV), Clover yellow vein virus, Bean common mosaic virus, Peanut mottle virus and Bidens mottle virus, can all infect lupins (Sweetingham et al. 1998). CMV and BYMV are the two major viral pathogens of lupin. Both can cause great yield loss under favourable conditions such as rainfall, adjacent alternative host plantation and high aphid population (Thomas et al. 2008a).

CMV is a seed-borne virus that infects *L. angustifolius* and *L. luteus* but does not infect *L. albus* and some other lupin species such as *L. cosentinii* and *L. pilosus* (Sweetingham et al. 1998). The main infection source for lupin crops is sowing infected lupin seed (Jones et al. 2010b).

BYMV is seed-borne in *L. albus*, *L. luteus*, *L. pilosus* and *L. atlanticus* but infection of *L. angustifolius* is mainly through infected alternative hosts (Sweetingham et al. 1998). In contrast, all endemic BYMV strains in south-western Australia, including both the common necrotic strain and the less abundant non-necrotic strain, are not seed-borne in lupins (Jones et al. 2010a).

Both CMV and BYMV are spread by many aphid species including the three main species listed in Appendix 3, in a non-persistent manner (Pirone & Harris 1977).

7.3 Other biotic interactions

Like other legumes, lupins can fix atmospheric nitrogen through the formation of root nodules, the highly specialised organs that result from the symbiosis between the host plant and the soil rhizobia. The genus *Lupinus* is nodulated by the rhizobial species *Bradyrhizobium* sp. (*Lupinus*) (Kurlovich et al. 2002c). *Bradyrhizobium* adapts well to acid soils (pH below 6.5) but its symbiosis with lupin may be impaired on alkaline soils (Tang & Robson 1995). In Australia, as a general practice, all lupins sown in a paddock for the first time should be inoculated with *Bradyrhizobium* sp. (*Lupinus*) inoculant. A lupin crop does not need to be inoculated for five years on acid soils once a well nodulated lupin crop has been grown, but seeds need to be inoculated every time a lupin crop is grown on neutral or alkaline soils (Thomas et al. 2008b).

SECTION 8 WEEDINESS

8.1 Weediness status on a global scale

As discussed in Section 1, the *Lupinus* genus is widely spread around the world with forms that range from annual and perennial herbaceous species to some shrubby or tree types. The geographical distribution of some major lupin species is outlined in Appendix 1. The USDA-

NRCS plant database contains 165 lupin species (USDA-NRCS 2012) and none of them has been included in the USDA Invasive and Noxious Weeds list (USDA-NRCS 2010). Holm et al. (1979) listed 16 lupin species as weeds in countries including Chile, Morocco, New Zealand, Spain and the USA. Globally, Randall (2002) named thirty-eight lupin species as weeds. Among them, *L. arboreus* Sims and *L. argenteus* Pursh were listed as noxious weeds in North America and *L. arboreus* was also listed as a quarantine weed in WA. In New Zealand, the introduced Russell lupin (*L. polyphyllus*) is also a well-known weed (Harvey et al. 1996).

Apart from possibly being weedy in their natural habitat, lupins may also be able to depress native plant species by altering soil characteristics through their nitrogen fixation and allowing the spread of non-native species (Adair & Groves 1998). For example, yellow bush lupin (*L. arboreus*), which is present in areas of the United States, Canada, France and Argentina, has been shown to promote weed invasion by increasing nitrogen levels and creating bare ground (Maron & Connors 1996), and has enhanced the spread of exotic weeds in the once nitrogen deficient Northern California sand dunes (Pickart et al. 1998).

However, some lupin species, such as Kincaid's lupin (*Lupinus sulphureus* ssp. *Kincaidii*) and Scrub lupin (*Lupinus aridorum*), are also listed as threatened or endangered plant species in the USA (<http://www.fws.gov/endangered>) for protection.

8.2 Weediness status in Australia

As discussed in Section 2.3, lupins are not native to Australia. Some lupin species, including *L. albus*, *L. angustifolius*, *L. cosentinii*, *L. luteus* and *L. pilosus*, were introduced to Australia for agricultural purposes. Other lupin species such as *L. polyphyllus* Lindley and *L. arboreus* were introduced as ornamental plants (Groves et al. 2005). As shown in Table 7, various lupin species have escaped from agriculture or gardens and become naturalised in all states except for the Northern Territory.

Table 7. Distribution of naturalised lupins in Australia*

Species	Cultivated	States where naturalisation occurred					
		NSW	QLD	SA	TAS	VIC	WA
<i>L. albus</i>	Yes		√			√	√
<i>L. angustifolius</i>	Yes	√	√		√	√	√
<i>L. arboreus</i> Sims	Ornamental				√	√	
<i>L. cosentinii</i> Guss.	Yes	√	√	√			√
<i>L. luteus</i>	Yes	√		√			√
<i>L. pilosus</i>	Yes	√					√
<i>L. polyphyllus</i> Lindl.	Ornamental	√		√		√	

*Source: (DPI Victoria 2009; Richardson et al. 2011)

8.2.1 Weediness in agricultural ecosystems

Among the naturalised lupin species, *L. angustifolius*, *L. albus* and *L. cosentinii* are considered major weeds in Australian agricultural ecosystems, particularly in WA, while *L. luteus* and *L. pilosus* are minor weeds that warrant control (Groves et al. 2003). *L. arboreus* and *L. polyphyllus* are not recorded as agricultural weeds.

As discussed in Section 2.3, the domesticated, white-flowered *L. angustifolius* is the dominant species for lupin production in Australia. All cultivars of this species are soft-seeded with little dormancy compared to the wild blue-flowered counterpart, which is hard-seeded with prolonged dormancy (Boersma et al. 2007a). This greatly reduces the weediness potential of the lupin cultivars, particularly in crop rotation systems involving lupin.

8.2.2 Weediness in natural ecosystems

L. angustifolius, *L. arboreus*, *L. cosentinii* and *L. polyphyllus* are generally considered significant weeds in Australian natural ecosystems. However, none of them are recorded as controlled or noxious weeds (Groves et al. 2003). *L. cosentinii* is a significant environmental weed in WA but not regarded as serious problem in other parts of Australia (DEEDI 2011a). Although not widely naturalised in Australia, *L. arboreus* is regarded as a significant weed in the coastal regions in Tasmania and Victoria, and thought to pose a serious environmental threat to coastal dunes (DEEDI 2011b). *L. albus* and *L. pilosus* are minor weeds that are not considered important enough to warrant control. *L. luteus* is also a minor weed but warranting control (Groves et al. 2003).

In WA, *L. cosentinii* is a widespread weed of roadsides, woodlands and heath from Carnarvon to Esperance, while *L. angustifolius* is a weed of road verges and woodlands from Geraldton to Albany. *L. luteus* can be found on roadsides and wasteland between Perth and Albany. *L. albus* is occasionally found on the Swan Coastal Plain and cropping belt (Hussey et al. 2007).

8.3 Control measures

In agricultural systems, lupin volunteers can be controlled through prevention of seed set for 3-4 years by mowing, grazing, cultivating and spraying with herbicides or hand pulling before flowering.

Herbicides (individual or in combination) in groups B, C, F, G, H, I and O can be used to control lupin volunteers either pre-emergence or post-emergence (Stewart et al. 2012). A number of selective herbicides for broadleaf weeds provide good control of lupin. These include Lontrel 750 or Transit 750 (active ingredient: clopyralid), Logran (active ingredient: triasulfuron) and X-Pand (active ingredients: florasulam and isoxaben) (Dow AgroSciences 2009; HerbiGuide 2012). Clopyralid based herbicides are particularly effective on members of the legume family (Tu et al. 2001). The non-selective glyphosate herbicides are relatively ineffective on lupins (HerbiGuide 2012).

In Australia, lupins in triazine tolerant canola are not well controlled with pre-or post-emergent atrazine application only. However, the addition of Lontrel or Dicamba has been shown to be effective in controlling lupin volunteers (Piper 1998).

In non-managed environments in Australia, grazing by native animals usually keeps lupins under control in healthy bushland (HerbiGuide 2012).

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

9.1 Intraspecific crossing

Annual lupins, which include all cultivated lupin species, are generally self-pollinating, although outcrossing can occur at a low rate with variation within and among different species (see Section 4). The rate of intraspecific crossing of lupins is determined by several factors including the outcrossing behaviour of the varieties, spatial distribution, relative flowering times and the absence or presence of pollinating agents (such as bees) (Hamblin et al. 2005). The outcrossing behaviour of a particular lupin variety is associated with the development and opening of the anthers in relation to the opening of the flower.

Under field conditions, different crop lupin species display different outcrossing ability. *L. albus* has an outcrossing rate of 5-10 % (Lin et al. 2009; Luckett 2010). Adhikari et al. (2006) studied the outcrossing rate in *L. luteus* in a small scale (300 m × 20 m) experiment by using two genotypes planted adjacent to each other, one with the orange flower colour (controlled by a single dominant gene) and the other one with lemon flower colour. They found that up to 8 % outcrossing occurred within 4 m with the presence of honeybees, and no outcrossing was observed beyond 25 m.

In *L. angustifolius*, the level of outcrossing is determined by factors such as genotype, flowering times, spatial separation and bee activity. Reported natural cross-pollination between narrow-leafed lupin plants in close proximity ranges from 0–0.4% in one study (Wallace et al. 1954) and 0–2.3% in another study with a blue-flowering genotype showing higher outcrossing rate than the white-flowering genotype (Dracup & Thomson 2000). Cross-pollination generally falls with distance and was recorded at 0.1% at 5 m (Quinlivan 1974). In another larger scale field trial, Hamblin et al. (2005) assessed pollen flow from a plot of GM line carrying the *bar* gene to surrounding non-GM parental plants, separated by 0.75 m of bare ground. They observed an outcrossing rate of 0.028 % in approximately 1.56 million seeds collected from the first 1.5 m of non-GM plants closest to the GM plants. In addition, no outcrossing was detected from smaller seed samples (almost 5000 seeds per sample) collected from 1 m² quadrats at distances of 0, 2.5, 5, 10, 20, 40, 80, 160 and 320 m into non-GM plants along the four cardinal points of the compass.

9.2 Natural interspecific crossing

Species within the genus *Lupinus* have cytogenetic barriers which prevent interspecific hybridization; and the formation of viable hybrids is extremely difficult (Wolko et al. 2011; Zoga et al. 2008). Such barriers are more prevalent in the Old World lupins than the New World ones due to a more diverse number of chromosomes and greater phylogenetic distance among the Old World lupin species (see Section 1).

The chromosome numbers in the four cultivated Old World species are: *L. angustifolius* 2n = 40, *L. albus* 2n = 50, *L. luteus* 2n = 52 and *L. cosentinii* 2n = 32 (Sawicka-Sienkiewicz et al. 2008; Wolko et al. 2011). Although the New World species have the widest diversity in terms of ecological distribution, the majority of the examined species have the chromosome number 2n = 48. The Andean lupin *L. mutabilis* (2n = 48) is the only cultivated New World species (Sawicka-Sienkiewicz et al. 2008). The formation of viable hybrids among these five cultivated species under natural conditions has not been reported in published literature.

According to Bevan Buirchell (personal communication, 16 October, 2012), interspecific lupin hybrids do not form in nature, and if they did, they would not be viable.

9.3 Crossing under experimental conditions

Even under the experimental conditions, interspecific hybrids within the genus *Lupinus* have been difficult to obtain (Sawicka-Sienkiewicz et al. 2006; Wolko et al. 2011). Since the New World lupins share the predominant chromosome number ($2n = 48$), many attempts to obtain interspecific hybrids among the species within this group have been made. Old World lupin species are a much less homogeneous, more widely separated group; interspecific hybridisation among them or between them and the New World species has been extremely difficult due to their different chromosome numbers (Wolko et al. 2011). Data regarding hybrids presented in this section were obtained in experimental settings with crosses by emasculation and hand pollination either under field conditions or in glasshouses. Some viable hybrids may only be obtained with the aid of embryo rescue techniques (Wilson et al. 2008). The recent development of male-sterile plants in *L. angustifolius*, *L. luteus* and *L. mutabilis* may facilitate future interspecific hybridisation programs (Clements et al. 2012).

9.3.1 Crossing among New World species

Early studies on crossability between lupin species focused on garden lupins such as Russell lupin *L. polyphyllus* Lindl., yellow bush lupin *L. arboreus* Sims. and Nootka lupin *L. nootkatensis* Donn. Russell lupin is thought to be a hybrid between *L. arboreus* and *L. polyphyllus*, and hybrids can be obtained from crosses between Nootka lupin and Russell lupin or yellow bush lupin without embryo rescue (Bragdø 1957).

Recent studies of interspecific crossing among the New World species have mainly been centred on the only crop species in this group, *L. mutabilis*. Attempts at hybridisations between *L. mutabilis* and other New World species have produced some successful cross combinations, as listed in Table 8. Clements et al. (2008) also showed that crossing success depended on directions of the crosses. For example, viable seed can be obtained from *L. hartwegii* (female) \times *L. mutabilis* (male), but in the reciprocal cross, hybrid plant can only be obtained through embryo rescue. Viable seed can be obtained from *L. mutabilis* (female) \times *L. tomentosus* (male) but not in the reciprocal direction.

Table 8. Hybridisations between *L. mutabilis* and other New World species*

Female	Male	Stage achieved	Embryo rescue
<i>L. elegans</i>	<i>L. mutabilis</i>	Viable seeds	No
<i>L. polyphyllus</i>	<i>L. mutabilis</i>	Viable plants	No
<i>L. pubescens</i>	<i>L. mutabilis</i>	F1 seeds	No
<i>L. nanus</i>	<i>L. mutabilis</i>	F1 seeds	No
<i>L. hartwegii</i>	<i>L. mutabilis</i>	Viable seeds	No
<i>L. mutabilis</i>	<i>L. hartwegii</i>	F2 seeds	Yes
<i>L. mutabilis</i>	<i>L. tomentosus</i>	Viable seeds	No

*(Clements et al. 2008; Clements et al. 2005a)

9.3.2 Crossing among Old World species

Successful crosses between Old World species were achieved first among rough-seeded lupins using specially selected lines. Successful crosses include partially fertile F₁ plants between *L. palaestinus* and *L. pilosus* (both $2n = 42$) (Pazy et al. 1981); partially fertile F₂

plants between *L. atlanticus* ($2n = 38$) and *L. digitatus* ($2n = 36$) or *L. cosentinii* ($2n = 34$) (Roy & Gladstones 1985) and between *L. digitatus* and *L. cosentinii* (Roy & Gladstones 1988); and viable F_2 seeds between *L. digitatus* and *L. cosentinii* or *L. atlanticus* (Gupta et al. 1996). Therefore, the most mutually compatible species in crossability are *L. digitatus*, *L. cosentinii* and *L. atlanticus*.

Among the smooth-seeded species, *L. luteus* and *L. hispanicus* have the same chromosome number ($2n = 52$). Crosses between them can produce fertile hybrids without embryo rescue (Swiecicki et al. 1999).

Because the smooth-seeded crop lupin species, including *L. angustifolius*, *L. albus* and *L. luteus*, are a much less homogeneous group due to the phylogenetic distance, interspecific hybridisation among them or between them and the rough-seeded species or the New World species (eg *L. mutabilis*) has been very difficult (Wolko et al. 2011). Kasten et al. (1991) obtained some F_1 plants from crosses between *L. angustifolius* and *L. luteus* using an embryo rescue technique. However, these plants did not survive after being transferred to soil.

In a recent study, Clements et al. (2009b) crossed approximately 5400 flowers in combinations of *L. angustifolius* × *L. luteus*, *L. angustifolius* × *L. albus*, *L. angustifolius* × *L. mutabilis*, *L. luteus* × *L. mutabilis* and *L. albus* × *L. mutabilis*, including the reciprocal crosses. They confirmed that hybrid embryos did develop from crosses between *L. angustifolius* and *L. luteus* when *L. angustifolius* was used as the female parent, and obtained flowering F_1 hybrid plants with intermediate morphological characteristics using embryo rescue methods. However, specific genotype combinations need to be used to produce hybrid embryos.

9.3.3 Intergeneric crossing

There are 730 genera (about 180 in Australia) in the Fabaceae family (Richardson et al. 2011). Hybridisation between species of *Lupinus* and species of other genera under either natural or experimental conditions has not been reported.

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APPENDICES

Appendix 1. The geographical distribution of major lupin species *

		Species	Synonyms	Common name	Distribution
Old World	Smooth-seeded	<i>L. angustifolius</i>	<i>L. linifolius</i> Roth., <i>L. varius</i> , <i>L. reticulatus</i> Desv., <i>L. opsianthus</i> Atab. & Maiss.	Narrow-leaved lupin, Blue lupin(e)	Pan Mediterranean, particularly Iberian Peninsula; Widely cultivated in Australia
		<i>L. albus</i>	<i>L. albus</i> subsp. <i>albus</i> , <i>L. albus</i> var. <i>termis</i> (Forsk.) Caruel	Albus lupin, White lupin, Lupino, Weisse	Pan Mediterranean
		<i>L. luteus</i>		Yellow lupin, Gelbe Lupine, Altramuz Amarillo	West Iberia; pan-Mediterranean
		<i>L. hispanicus</i>	<i>L. rothmaleri</i> Klink.		Central, South, North-west Spain; Portugal; Greece; Turkey
		<i>L. micranthus</i>	<i>L. hirsutus</i>		Circum-Mediterranean
	Rough-seeded	<i>L. pilosus</i>	<i>L. hirsutus</i> , <i>L. varius</i> , <i>L. varius</i> spp <i>orientalis</i> , <i>L. anatolicus</i> Swiec.		North-east Mediterranean
		<i>L. cosentinii</i>	<i>L. hirsutus</i> Black, <i>L. digitatus</i> Lojac., <i>L. pilosus</i> ssp <i>cosentinii</i> , <i>L. varius</i>	Sandplain lupin, Western Australian blue lupin	West Mediterranean, Morocco, Australia naturalized
		<i>L. digitatus</i>	<i>L. tassilicus</i> Maire, <i>L. semiverticillatus</i> Desr.		Africa – Sahara, Egypt
		<i>L. princei</i>			East Africa
		<i>L. palaestinus</i>			South-east Mediterranean, Middle-east
		<i>L. atlanticus</i>		Atlas lupin, Moroccan lupin	South Morocco
		<i>L. somaliensis</i>			Somalia
	New World	<i>L. mutabilis</i>		Andean lupin, Pearl lupin, tarwi, chochos, tauri, chuchus, ccequla, ullush	Ecuador, Peru, Bolivia (Andean region 2000 – 4000m)
		<i>L. polyphyllus</i>		Large-leaved lupin, Blue-pod lupin, Garden lupin, Washington lupin, Russell lupin	Ecuador, Peru, Bolivia (Andean region 2000 – 4000m)
<i>L. texensis</i>			Texas Blue Bonnet	Southern USA	
<i>L. arboreus</i>			Yellow Bush lupin, Coastal Bush lupin, Tree lupin	California coast, Western North America, naturalized elsewhere eg. Southern England, New Zealand	
<i>L. nootkatensis</i>			Nootka lupin	Canada - British Columbia, Yukon Territory; Alaska, Iceland (naturalized)	
<i>L. argenteus</i>			Silvery lupin	South-western Canada to South-western USA	

*Sources: (Gladstones 1998; Information portal for lupins 2010a; USDA-NRCS 2012)

Appendix 2. Lupin varieties suitable to be sown in Australia *

Species	Variety	Maturity	Anthraco- nose resistance	Aphid resistance	Brown spot resistance	Metribuzin tolerance	Grain protein	PBR in Australia ^a	PBR ^b Owner	Marketed by
<i>L. angustifolius</i>	Belara	Very Early	Intermediate	High	Low	Intermediate	Low	No		
	Coromup	Early	High	High	Intermediate	High	High	Yes	DAFWA	Coorow Seeds
	Jenabillup	Very Early	Intermediate	High	Intermediate	Low	Intermediate	Yes	DAFWA	The Seed Alliance Group
	Jindalee	Late	Low	High	Intermediate	Intermediate	Intermediate	Yes	NSW DPI	AWB Seeds
	Kalya	Early	Intermediate	High	Intermediate	High	Intermediate	No		
	Mandelup	Very Early	High	High	Low	High	Intermediate	Yes	DAFWA	Vitera/PlantTech
	Merrit	Early	Intermediate	High	Intermediate	High	Intermediate	No		
	Moonah	Early	Intermediate	High	Intermediate	Intermediate	Intermediate	Yes	DAFWA	AWB Seeds
	Myallie	Early	Low	Low	Intermediate	Intermediate	High	No		
	PBA Gunyidi	Very Early	High	High	Low	High	Intermediate	Yes	PBA	SeedNet
	Quilinock	Early	Very Low	Low	Intermediate	Intermediate	Intermediate	Yes	DAFWA	PlantTech
	Tanjil	Early	Very High	High	Intermediate	Low	Intermediate	Yes	DAFWA	PlantTech
Wonga	Early	Very High	High	Intermediate	Low	Intermediate	Yes	NSW DPI	Seedmark	
<i>L. albus</i>	Andromeda	Late	Intermediate	High	Low	Low	High	Yes	DAFWA	Coggo Seeds
	Kiev Mutant	Mid	Very Low	High	Low	Low	High	No		
	Luxor	Late	Very Low	High	Intermediate	Low	High	Yes	NSW DPI	Viterra Seeds
	Rosetta	Late	Very Low	High	Intermediate	Low	High	Yes	NSW DPI	Viterra Seeds
<i>L. luteus</i>	Pootallong	Early	Low	Very Low	High	Low	Very High	?		
	Wodjil	Early	Low	Very Low	High	Low	Very High	Yes	DAFWA	DAFWA

a, Proprietary plant varieties in Australia are protected by Plant Breeder's Rights (PBR), which are exclusive commercial right to a registered variety. The rights are a form of intellectual property and are administered under the Plant Breeder's Right Act 1994 (refer to IP Australia website at <http://www.ipaustralia.gov.au/pbr/index.shtml> for more information).

b, DAFWA, Department of Agriculture and Food Western Australia; NSW DPI, New South Wales Department of Primary Industry; PBA, Pulse Breeding Australia.

*Sources: (GRDC 2010; Information portal for lupins 2010d; Ware & Hawthorne 2012; Wheeler & McCormack 2010)

Appendix 3. Common invertebrate pests of lupin in Australia*

Pest	Common name	Taxonomic name	Impact	
Seedling stage	Caterpillars	Cutworms	<i>Agrotis</i> spp.	Chews through stems at ground level; occurs sporadically causing patches of bare ground
		Brown pasture looper	<i>Ciampa arietaria</i>	Chews cotyledons and first leaves; occurs sporadically causing patches of bare ground
	Springtail	Lucerne flea	<i>Sminthuris viridis</i>	Feeding on leaves resulting in white windows (holes); occurs occasionally
	Mites	Red-legged earth mite	<i>Halotydeus destructor</i>	Rupturing cells and sucking on young seedlings leading to a leathery and silvery appearance of the leaves; Lupin crops can usually grow away from the damage
		Blue oat mite	<i>Penthaleus</i> spp.	Same as red-legged earth mite
		Balaustium mite	<i>Balaustium medicagoense</i>	Causes a leathery, silvered appearance of cotyledons and leaves; Lupin crops can usually grow away from the damage
		Clover mite (Bryobia mite)	<i>Bryobia praetiosa</i>	Same as red-legged earth mite
Fly	Bean seedling maggot	<i>Delia platura</i> (Meigen)	Feeding on hypocotyl and tap root; occasionally causes loss in <i>L. angustifolius</i>	
Slugs	Black keeled slug	<i>Milax gagates</i>	Chews leaves or whole seedlings; Ten large slugs/m ² may destroy an emerging crop	
	Reticulated slug	<i>Derocerus reticulatum</i>		
Snails	Small pointed snail	<i>Cochlicella barbara</i>	Cause very similar damage to slugs	
	White Italian snail	<i>Theba pisana</i>		
	Vineyard snail	<i>Cernuella virgata</i>		
Vegetative and reproductive stages	Aphids	Green peach aphid	<i>Myzus persicae</i>	High numbers sucking on young leaves and buds may cause wilting and abortion of flowers and young buds; Vectoring viral diseases
		Blue green aphid	<i>Acyrtosiphon knodoi</i>	
Cowpea aphid		<i>Aphis craccivora</i>		
Thrips	Onion thrips	<i>Thrips tabaci</i>	sucking on young leaves and buds may produce distorted leaves and cause flower abortion; Economic damage to crops has been rare	
	Plaque thrips	<i>Thrips imagines</i>		
Pod and seed stages	Caterpillars	Native budworm	<i>Helicoverpa punctigera</i>	Penetrated pods and eaten seeds
		Lucerne seed web moth	<i>Etiella behrii</i>	Penetrated pods and eaten seeds; caused little damage to seed in most seasons but significant yield losses have been reported
	Mirid	Mirid bugs	<i>Lygus</i> spp.	Sucking resulting in abortion of young pods, no economic damage reported

* Sources: (Mangano et al. 2008; Sweetingham et al. 1998)

Appendix 4. Common lupin diseases in Australia*

	Diseases	Pathogen	Symptoms	Impact
Fungal diseases	Pleiochaeta root rot; brown leaf spot	<i>Pleiochaeta setosa</i>	Browning and rotting of root; Brown-black spots on leaf, stem and pod	Plant vigour reduced or seedlings killed leading to decreased plant density; may reduce yield potential by 20% - 40%
	Rhizoctonia bare patch (strains ZG1, ZG2); Rhizoctonia hypocotyl rot (strains ZG3, ZG4); Rhizoctonia root and hypocotyl rot (strain ZG6)	<i>Rhizoctonia solani</i>	Browning and rotting of root; Red-brown sunken lesions on hypocotyl and root	Bare patches with distinct edges; Yield loss from poor vigour and death of seedlings; Plant establishment could be reduced by 80% if severely affected
	Eradu patch	<i>Rhizcotonia</i> sp.	Red or brown lesions on root; Nodulation reduced	Specific to <i>L. angustifolius</i> ; yield loss depending on the severity of the infection
	Anthracnose	<i>Colletotrichum lupini</i>	Lesions containing pink-orange spores twisting, distorting or severing stem, petiole and pod	Important in warm and wet regions
	Phomopsis	<i>Diaporthe toxica</i> (formerly known as <i>Phomopsis leptostromiformis</i>)	Grey to purple lesions on stems and golden-brown seed	Very common; the mycotoxin produced by the fungus can cause lupinosis in stock
	Sclerotinia collar rot	<i>Sclerotinia minor</i>	White, cottony growth on lower stem and upper root	Minor
	Sclerotinia stem rot	<i>Sclerotinia sclerotiorum</i>	White fungal growth on upper stem or branches	Minor
	Charcoal rot	<i>Macrophomina phaseolina</i>	Stem base and taproot gray-coloured	Limited to periods of water stress
	Gray leaf spot	<i>Stemphylium vesicarium</i>	Small gray circular lesions on leaf and pod	Not important now due to resistance of commercial lupin varieties
	Cladosporium leaf spot	<i>Cladosporium</i> sp.	Dark-grey spots on leaf	Minor
	Grey mould	<i>Botrytis cinerea</i>	Large lesions girdling stems and branches; abortion of flower and pod	Minor
	Powdery mildew	<i>Erisphe polygoni</i>	White powdery fungal growth on leave, stem and pod	Minor
Viral diseases	Cucumber mosaic	Cucumber Mosaic Virus	Plants stunted with pale leaves bunched and turned down	Capable of causing up to 60% yield loss; Transmitted by aphids
	Bean yellow	Bean Yellow Mosaic Virus	Brown streaks moving away from shepherds crook at growing tip, associated with leaf yellowing	Most important viral disease of lupins affecting all commercial species; Transmitted by aphids

*Sources: (Information portal for lupins 2010d; Sweetingham et al. 1998; Thomas et al. 2008a)