Department of Agriculture
Government of Western Australia

# THE AUSTRALIAN LUPIN COLLECTION
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# • Summary

Although its history is traceable back to the early 1920's, the Minister for Agriculture, the Hon Monty House MLA officially opened the Australian Lupin Collection on April 13 1994.

The collection remains an integral part of lupin breeding in southern Australia. Initially located at the University of Western Australia during the 1960's & 70's, it transferred in 1972 to the Western Australian Department of Agriculture.

Accessions in the collection consist of wild, semi-domesticated, domesticated, fixed lines, mutants and cultivars of over thirty lupin species. The seed came from collectors, gene banks and breeders from around the world to make it, arguably, one of the most comprehensive collections of 'Old World' lupins. The resource provides a broad base for future lupin improvement and is an essential tool for Australian lupin breeders in their quest for higher yield, greater disease resistance and better adaptation to the farming systems of southern Australia.

For the 10 years since the collection was formally established, I have had the responsibility of managing it.

Dr Jon C Clements was responsible for assembling the collection as part of a research project commencing in 1988 (Clements and Cowling 1993). He also collated passport, accession and evaluation data on a mainframe database using Henco *INFO®*. That data moved to a personal computer database (Microsoft Access ®) in 1993. Passport, collection site and evaluation data where available are added to the database and the results published on the internal and external website of the Department of Agriculture (http://www.agric.wa.gov.au/)

The collection housed here at South Perth is a breeders or 'active' collection and also a base (i.e. long-term storage) set of the collection is stored at the Australian Temperate Field Crops Collection (ATFCC) in Horsham, Victoria. The ATFCC is a part of the Australian Network of Plant Genetic Resources.

The collection continues to increase in numbers and at December 2003 contained 3,639 entries. Wild and landrace lines constitute 72% of the collection; the rest being fixed breeding-lines, cultivars and mutants. Over thirty species of lupin are in the collection, the majority being 'Old World' annuals with some perennials from the Americas. Some 600 lines are in post-entry quarantine waiting processing and later addition to the collection.

It is now over 10 years since the Australian Lupin Collection (ALC) moved to its present location in Building 58 at the Western Australian Department of Agricultures' (WADA) South Perth site.

This report covers the history of the collection, reports on its current activities, and makes recommendations for the future.

## 1. History of the Australian Lupin Collection

The first entries collected for the Australian Lupin Collection originated from the 1920's when two officers of the Department of Agriculture, F L Shier and I Thomas conducted yield trials at the Merredin and Chapman Valley Research Stations. The seed for those trials came from Germany in 1920 and later, went to the Wongan Hills Research Station for storage. In 1954 that seed became the basis for an Honours study by Dr J S Gladstones to evaluate the usefulness of lupins to Western Australian agriculture. The work expanded to a PhD project and resulted in the release to WA farmers in 1959 of the German Yellow lupin (*L. luteus* L.) variety Weiko III and the Swedish narrow-leafed lupin variety Borre (Gladstones 1994).

Naturalised populations of wild and landrace lupins occur in southern Western Australia and many are in the ALC. Two species dominate the local populations. They are the sand plain lupin *L. cosentinii* Guss. and the narrow-leafed lupin (NLL) *L. angustifolius* L.; both probably introduced in stock feed sometime in the early to mid 19th century (Gladstones 1970). Additionally, three other species of lupin are naturalised in southern WA; they are *L. luteus* L, *L. pilosus* L and *L. albus* L and with the other two, account for over 70 entries in the ALC.

A lupin-breeding program conducted by Dr J S Gladstones to fully domesticate both the NLL and *L. cosentinii* commenced in 1960 at the University of Western Australia. He had earlier collected lupins from around the southwest of WA to use in the breeding program and added many entries of interest to the collection. To further expand the genetic diversity Dr Gladstones went to southern Italy in 1968; North Africa, Spain and Portugal in 1973; France in 1978 and Greece in 1984 (Gladstones 1973, 1974, 1976, Gladstones and Crosbie 1979). These trips were highly successful and contributed an enormous number of lines to the collection. Dr Gladstones dominates the list of contributors as shown in Table 1.

As the collection continued to grow, the need arose to better document and conserve it in order to make the best use of the resource. Dr J C Clements commenced a research project in 1988 with the main aims being to assemble, evaluate, document, utilise and ensure the conservation of the resource. That project was funded initially by the then Grain Legume Research Council, the Western Australian Department of Agriculture, the Grain Research Committee of WA and the Fourth International Lupin Conference Trust Fund (Clements and Cowling 1993).

The recommendations from that project formed the basis of a submission to the Centre for Legumes in Mediterranean Agriculture at the University of Western Australia (CLIMA) for additional funding to upgrade the storage and work facilities for the collection. The Grains Research and Development

Corporation through CLIMA provided that funding. The Australian Lupin Collection was later officially launched in 1994 by the then Minister for Agriculture, the Hon Monty House MLA.

Although not part of the Australian Network of Plant Genetic Resources, seed is sent to the ATFCC for storage as the lupin base-collection.

Since 1993, I have managed the ALC using the system as outlined by Clements and Cowling (1993) in their final report to the GRDC. Some variations to their model have evolved because of constraints in the availability of storage and regeneration space. Other techniques they suggested in maintaining the veracity of the collection are utilised. For example, viability testing of all seed placed in the collection is now routine.

Figure 2 shows an overview of the system used to manage the ALC.

Many other collaborators from the WA Department of Agriculture (WADA) and elsewhere continue to add accessions to the collection. Donations from genebanks around the world have enhanced it to its current number of over 3600 accessions. About 2600 are designated as wild or landrace; the rest are cultivars, mutants or semi-domesticated types. The most numerous species is *L. angustifolius* L., accounting for nearly 55% of the collection.

Over the next year or so, the collection is likely to expand rapidly as a further 600 lines of various species are in quarantine, waiting post-entry regeneration.

The storage in the ALC of marker gene populations currently under production will also add significant numbers to the collection.

Statistical information about the collection is shown in Section 3 of this report.


**Figure 1: Compactus storage used for the Australian Lupin Collection**

## 2. Role and Methods Used

The main role of the Australian Lupin Collection is to conserve the genetic material in the collection for use by lupin breeders and researchers. That role includes the documenting, evaluating, regenerating and storing of the seed in the ALC. Figure 2 shows the current schema used by the ALC to fulfil that role in conserving the collection.

International standards and/or recommendations for the conservation of seed are followed where applicable (Painting et al 1995, Hong et al 1996 and Sackville Hamilton 2002).

Seed regeneration of accessions in the ALC occurs in insect-proof screen houses. This method is used because of the need to produce virus-free seed, but restricts seed production in late flowering lines due to the lack of vernalisation and the short season generated by the hot conditions in the screenhouse. Vernalising late flowering lines at 8$^{o}$C for three weeks before sowing to some extent overcomes this problem. Production of 2,000 seeds per line is desired and in most cases achieved. The average numbers of seeds produced per row over the last five years from screen house regeneration has been 2,287. However, screenhouse space is limited and there is always a conflict between the need to grow a representative sample from accessions and minimum seed requirements.

Routine evaluation data is collected from all regeneration rows. In all, 10 morphological and quantitative characters are recorded. These include height at 9 weeks, alkaloid status, seed size and flowering time. All data is posted to the ALC database and published on the DAWA web site.

Cucumber mosaic virus (CMV) contaminated much of the *L. angustifolius* seed grown before 1988. Therefore, regeneration priority is to replace seed suspected of having CMV. This need has diminished somewhat, because there is some evidence suggesting that the virus may die as the seed ages. In 1997, CMV occurred in 40% of the regeneration rows sown with pre-1988 seed. This figure has steadily declined and in 2003, no pre-'88 lines were found with CMV. Speculatively this suggests that the virus may have a limited life span in the seed. This hypothesis needs to be tested in order to make it clear wether pre-'88 seed should be kept.

Preliminary viability testing of seed in the ALC commenced in 1997 in order to establish priority for regenerating the collection. That testing has highlighted a problem with the post-harvest drying of the samples. Indeed, screen house drying, where the temperature is approximately 10$^{o}$C above ambient (Perth, November Average Maximum = 24.1 C (Gladstones, 1992)); can produce very low seed moisture contents (C Smith, unpublished). This in turn is a precursor to thresher radicle damage in some species (particularly *L. mutabilis*) because of thin seed coats (Clements *et al*, 2004, In Press). To avoid this problem, hand threshing is now the preferred method of pod threshing. Drying lupin seed below 5% moisture content (SMC) may not be a problem since some preliminary data suggests that germination remains reasonable for a short time, even at 0% SMC (C Smith, unpublished).

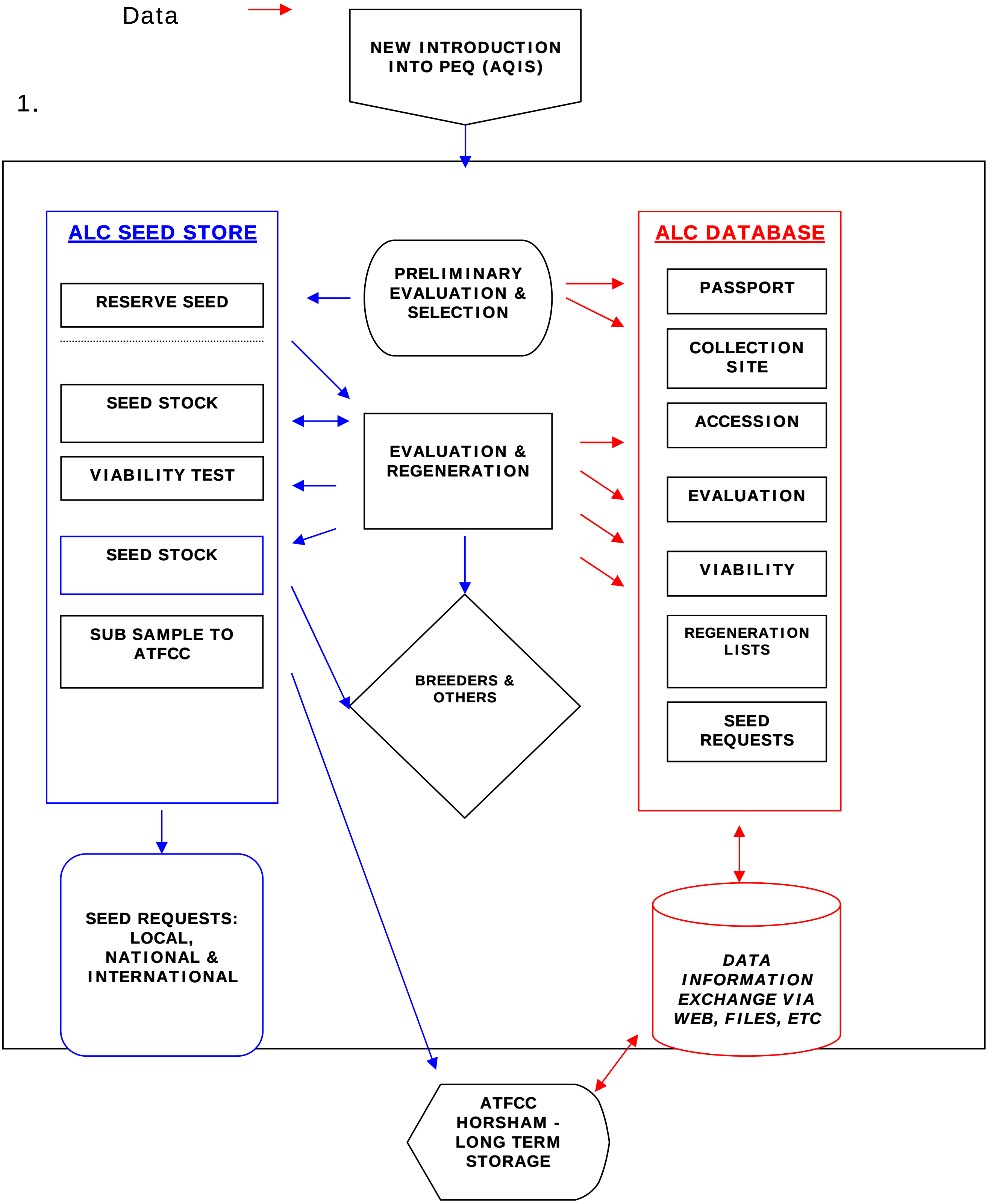
There is some evidence from viability testing that under current storage conditions, hard-seeded lines of *L. angustifolius* may retain satisfactory germination (>70%) well beyond 25 years (C Smith, unpublished).

A dehumidifier installed in the cool-room in mid-2003 has reduced the relative humidity from 75% to 10% rh, with the cool-room temperature set at 8$^{o}$C. These settings are similar to those used by the University of Illinios at Urbana, where the USDA *Glycine spp* collection is stored. See Figure 1 for a photograph of the cool room storage of the ALC.

# Figure 2. The Australian Lupin Collection Schema

Legend: Seed (blue arrow)
Data (red arrow)

1.



# 3. Collection Statistics

## 3.1 Accessions

Data in the tables below do not include accessions for which no seed is available from the ALC. Passport, accession, evaluation and site collection data for those lines however, remains in the database as suggested by Sackville Hamilton *et al* (2002).

Wild and landrace accessions (as defined by the GRIN Forage Legume dictionary, 2003) dominate the collection with over 72% being in those categories. Where a new accession's breeding status is unknown or is in doubt, classification occurs in the initial evaluation row. Lack of domestication traits helps determine the status of any line but not with complete certainty. "Landrace" on the other hand is defined by GRIN as: '...Accession is a cultivated species that has evolved from a wild population due to the selection pressure of traditional farmers as they have cultivated germplasm over time...' That definition may also refer to a primitive cultivar; that is, one not subject to modern plant breeding but having no or few 'wild' traits. This is best demonstrated in *L. albus* where of 873 lines listed, 674 or 77% are classed as Landrace types.

Table 3 lists the numbers of accessions in the collection designated as either Wild or Landrace. Wild types are further sub-divided into Introduced from outside Australia; 'Naturalised' found within Australia; and 'Selection from' selected from a regeneration row.

| BREEDING STATUS | Number of Accessions |
|---|---|
| Landrace | 738 |
| Wild (introduced) | 1830 |
| Wild (naturalised) | 56 |
| Wild (Selection from) | 2 |
| **TOTAL** | **2626** |

**Table 3: Number of accessions with Wild or Landrace Status.**

Table 4 shows the numbers for accessions in the ALC with Breeding, Cultivar, Interspecific or Mutant status. As can be seen, 'Cultivar' is further subdivided.

| BREEDING STATUS | Number of Accessions |
|---|---|
| Breeding Line | 559 |
| Cultivar (Aust) | 27 |
| Cultivar (foreign) | 126 |
| Cultivar (selection from) | 47 |
| Cultivar (uncertain) | 97 |
| Interspecific Form | 4 |
| Mutant | 153 |
| **TOTAL** | **1013** |

**Table 4: Number of accessions with 'other' Status**

Table 5 below lists the species currently found in the ALC. The largest group in the ALC is *L. angustifolius* (49%) as this is the species with the greatest commercial use in southern Australia. The drop from the 64% reported by Clements and Cowling (1993) being due to the difference due to the large number of *L. albus* entries added to the collection. *L. albus* provides 873 entries or 24% of the collection, making it the second most represented species in the ALC. Important landrace accessions from Egypt of *L. albus* were added to the collection in 1993 (Dr B J Buirchell) and Ethiopia in 1997 (Dr C M Francis).

| SP NAME | Number of Accessions |
|---|---|
| Interspecific cross | 4 |
| L. affinus | 1 |
| L. albifrons | 1 |
| L. albus L. | 873 |
| L. angustifolius L. | 1793 |
| L. arizonicus (S. Watson) S. Watson | 1 |
| L. atlanticus Gladst. | 89 |
| L. cosentinii Guss. | 217 |
| L. densiflorus | 1 |
| L. digitatus Forskål | 4 |
| L. exaltus | 1 |
| L. gibertianus | 1 |
| L. hirsutissimus | 2 |
| L. hispanicus Boiss. et Reuter | 69 |
| L. luteus L. | 224 |
| L. mexicanus Cerv. Ex Lag. | 6 |
| L. micranthus Guss. | 43 |
| L. microcarpus | 1 |
| L. mutabilis Sw. | 104 |
| L. nanus | 1 |
| L. palaestinus Boiss. | 10 |
| L. pilosus Murr. | 163 |
| L. polycarpus | 1 |
| L. polyphyllus Lindl. | 4 |
| L. princei Harms | 5 |
| L. rotundiflorus | 1 |
| L. sparsiflorus Benth. | 1 |
| L. subcarnosus | 1 |
| L. succulentus | 3 |
| L. texensis Hook | 1 |
| L. truncatus | 3 |
| Not identified | 10 |
| **TOTAL** | **3639** |

**Table 5: Number of Accessions for each Species**

## 3.2 Use and Evaluation

One of the main roles for the ALC is to supply seed to collaborators. Below is a table showing the numbers of samples sent to collaborators for each of the last 5 years.

As can be seen, the numbers sent varies considerably but the average is around 316. Seed numbers per line varies considerably also, but I limit requests to 100 seeds where possible. This is good genebank practice as suggested by Sackville Hamilton *et al* (2002) and follows a conservative approach to allow for limited seed quantities. It also emulates the policy of the USDA ARS National Genetic Resources Program where seed is freely available but supply is limited the 100 seed maximum.

| | Accessions |
|---|---|
| 99 | 117 |
| 00 | 564 |
| 01 | 477 |
| 02 | 184 |
| 03 | 239 |

**Table 6: Number of Accessions sent to Collaborators**

Below, tables 7 and 8 show evaluation characters for days-to-flower and 100 seed weight respectively. They are shown in order to compare the data now and that published in Clements and Cowling (1993, pp14).

| SP NAME | Number Lines | Average | Min | Max | SD |
|---|---|---|---|---|---|
| L. angustifolius L. | 1591 | 100 | 66 | 144 | 12 |
| L. albus L. | 817 | 89 | 52 | 151 | 18 |
| L. luteus L. | 222 | 104 | 69 | 150 | 16 |
| L. cosentinii Guss. | 159 | 93 | 59 | 134 | 12 |
| L. pilosus Murr. | 141 | 93 | 72 | 132 | 14 |
| L. mutabilis Sw. | 103 | 89 | 65 | 116 | 11 |
| L. hispanicus Boiss. et Reuter | 66 | 141 | 116 | 157 | 9 |
| L. atlanticus Gladst. | 60 | 106 | 85 | 126 | 10 |
| L. micranthus Guss. | 40 | 117 | 98 | 143 | 10 |
| L. palaestinus Boiss. | 9 | 90 | 81 | 103 | 6 |
| L. truncatus | 3 | 102 | 87 | 132 | 25 |
| L. digitatus Forskål | 3 | 99 | 92 | 103 | 6 |
| L. princei Harms | 3 | 144 | 135 | 150 | 8 |
| L. affinus | 1 | 129 | 129 | 129 | |
| L. polycarpus | 1 | 115 | 115 | 115 | |
| L. sparsiflorus Benth. | 1 | 93 | 93 | 93 | |
| L. arizonicus (S. Watson) S. | 1 | 96 | 96 | 96 | |
| L. succulentus | 1 | 100 | 100 | 100 | |
| L. texensis Hook | 1 | 102 | 102 | 102 | |
| L. mexicanus Cerv. Ex Lag. | 1 | 113 | 113 | 113 | |

**Table 7: Number of entries and flowering time mean, minimum, maximum and standard deviation for lupin species at Perth, WA**

| SP NAME | Number Lines | Average | Min | Max | SD |
|---|---|---|---|---|---|
| L. angustifolius L. | 1744 | 12.1 | 2.9 | 23.5 | 3.9 |
| L. albus L. | 872 | 32.7 | 11.2 | 85.5 | 8.3 |
| L. luteus L. | 224 | 10.5 | 5.3 | 17.4 | 1.9 |
| L. cosentinii Guss. | 217 | 18.9 | 5.8 | 26.6 | 3.4 |
| L. pilosus Murr. | 136 | 47.7 | 4.2 | 74.6 | 11.7 |
| L. mutabilis Sw. | 103 | 16.6 | 1.7 | 25.3 | 4.1 |
| L. hispanicus Boiss. et Reuter | 71 | 6.2 | 3.7 | 11.6 | 1.9 |
| L. atlanticus Gladst. | 68 | 30.2 | 18.0 | 50.4 | 7.6 |
| L. micranthus Guss. | 42 | 8.4 | 4.0 | 13.9 | 2.5 |
| L. palaestinus Boiss. | 10 | 23.6 | 19.0 | 27.0 | 2.8 |
| L. digitatus Forskål | 4 | 11.0 | 10.1 | 12.0 | 0.7 |
| L. mexicanus Cerv. Ex Lag. | 4 | 1.6 | 1.4 | 1.8 | 0.2 |
| L. truncatus | 2 | 0.6 | 0.4 | 0.9 | 0.3 |
| L. gibertianus | 1 | 1.0 | 1.0 | 1.0 | |
| L. polycarpus | 1 | 1.1 | 1.1 | 1.1 | |
| L. princei Harms | 1 | 33.1 | 33.1 | 33.1 | |
| L. succulentus | 1 | 2.1 | 2.1 | 2.1 | |

**Table 8: Number of entries and 100 seed weight for the mean, minimum, maximum and standard deviation of lupin species at Perth, WA**

# 4. Discussion

In his 1997/98 Project Proposal for Lupin Breeding (UAI) Dr W A Cowling, the then Senior Plant Breeder (Lupins), listed as Project Objective (iii):

" To collect, assess, document, and conserve lupin genetic resources for use by lupin breeders and collaborating scientists".

That objective remains relevant today and continues to be the prime argument for continuing the support for the Australian Lupin Collection.

Below is some discussion relating to urgent issues affecting the collection.

### *Storage Space*

As can been seen in Figure 1, the collection is housed in a cool room of 50 $m^2$ in volume with a compactus unit consisting of 16 compartments. Each compartment consists of 11 shelves; each shelf can hold 90 PET jars giving a maximum available space for 15,840 jars. As each accession receives a minimum of two jars, the current space is sufficient for 7,900 entries. Since the current accessions number less than 4000, space for another 3,900 entries is available. By discarding some old seed now backed up in jars 3, 4, 5 and 6 of some lines, new entries from the breeders, collectors and lines from the molecular marker populations will fit into the ALC.

### *Line Purity*

One of the biggest problems with the collection is that of line purity. Much of the collection originates from rows or plots grown in the open field in the 70's and 80's and because of outcrossing, harvest contamination and/or cleaning contamination, much of it contains mixtures. Some of the 'mixtures' could also have originated from the original populations. Around 316 *L. angustifolius* lines have a seed description of "Mixed". Similarly, 44 *L. albus* and 29 lines of *L. luteus* have mixed seed lots.

J S Gladstones in his field notes on the 1974 and 1975 regeneration rows sown at the Medina Research Station using the original seed of the wild collections noted many lines were not pure.[1] This supports observations made by many other collectors of evolving or mixed populations.

[1] JS Gladstone's field notes are available at the ALC.

How does the ALC handle line purity problems?

Currently, I have a "keep all" policy when deciding what to retain in an accession. The evidence to discard an 'offtype' must be overwhelming and only in cases where it is clearly physical contamination, is then an offtype discarded. This system is as suggested by Sackville Hamilton et al (2002) and is to maintain the accessions genetic diversity until such time as economic or other reasons allow separation. Separation is conducted where a clear difference exists that may be of use to a breeder. All contamination or segregation for gross evaluation characters is noted in the field book and database.

Similarly, many lines in the collection may have duplicates, which came from collaborators or genebanks from several sources. I have continued to maintain the integrity of each accession since it is really due to local selection pressure, insights on genetically different lines will have evolved

***Future Management***

It is beyond the scope of this report to speculate on the future management of the ALC. However, the rapid access by breeders to the collection and others to the resource in the past has been a strong argument for keeping it at South Perth.

While the breeding of new lupin varieties remains primarily in Western Australia, then it could be argued that so should the ALC.

As with all genebank collections, the resource is finite and the temptation exists to use it to the maximum for studies of all descriptions. However, because of its very limited size, each accession must be treated with great care and decisions as to what use the accessions are put, must be weighed against the speed at which regeneration of the line is possible.

# 5. Acknowledgments

I thank Dr. Jon Clement, CLIMA for valuable discussion on the early drafts. Also thanks to Mr Jeff Beorsma, DAWA for this review.

I gratefully acknowledge the support and encouragement given to me by my supervisor Dr Bevan Buirchell. His support in allowing me professional creativity within this system is admiral.

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# APPENDICES:

Appendix 1: Collectors List

| Collector | Number of Entries |
|---|---|
| Adcock K G | 1 |
| Ainouche A | 8 |
| Andrada M da P Campos | 16 |
| Arnold G | 1 |
| Bailey E T * | 1 |
| Bateman D R | 2 |
| Behm | 1 |
| Boulos L | 1 |
| Bounejmate M | 20 |
| Broad M C | 1 |
| Buirchell B J | 190 |
| Clements J C | 3 |
| Collins W J | 9 |
| Corbin E * | 1 |
| Cowling K B | 1 |
| Cowling W A | 104 |
| Crosbie J | 1 |
| Curtin S | 1 |
| Downey R | 1 |
| Dracup M A | 3 |
| Drage B | 1 |
| Drossos E | 2 |
| Enneking D | 1 |
| Evans P | 7 |
| Ewing M A | 40 |
| Forbes I * | 27 |
| Francis C M | 106 |
| Gillespie D G | 26 |
| Gladstones J S | 391 |
| Hadjichristodoulou A | 3 |
| Halstead W R | 3 |
| Hamblin J | 19 |
| Heyn C | 10 |
| Horovitz A | 1 |
| Howieson J G | 5 |
| Jambrina J | 20 |
| Judges M | 19 |
| Kaess E | 1 |
| Katznelson J | 1 |
| Luebbermann S | 1 |
| Martins J M N | 6 |
| Mason G | 2 |
| McClements D R | 1 |
| McCormack J | 1 |
| Miles & Donald | 2 |
| Muehlbauer F J | 1 |

| | |
|---|---|
| Njarui D M | 2 |
| Nutt B | 62 |
| Oliver | 1 |
| Oram R N | 1 |
| Papineau | 3 |
| Parbery D | 1 |
| Parker R | 3 |
| Pazy B | 4 |
| Perrino P | 2 |
| Petterson D S | 1 |
| Pierce A | 1 |
| Plitman U | 5 |
| Pourpoutidis S | 1 |
| Price M | 1 |
| Pulawy | 1 |
| Quinlivan B J | 28 |
| Ralph C | 3 |
| Ramos Monreal A | 121 |
| Reid R | 6 |
| Robartson R | 1 |
| Robertson L D | 7 |
| Shea G | 3 |
| Simpson M J | 92 |
| Skinner P | 4 |
| Smith C G | 25 |
| Snowball R | 9 |
| Spencer D | 1 |
| Stern W R | 1 |
| Stretch W | 1 |
| Sweetingham M A | 3 |
| Timms (Kojonup, WA) | 1 |
| van Schoonhoven | 1 |
| Weeldenburg J R | 3 |
| **TOTAL** | **1462** |