

## The Sharnae greater lotus (*Lotus uliginosus* Schkuhr) germplasm – potential for low latitude environments

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### Summary

This paper is a review of the scientific literature and industry experience with the Sharnae greater lotus (*Lotus uliginosus* Schkuhr) germplasm in Australia. The purpose is to assess the merits and limitations of Sharnae based on knowledge and experience that was not available when Sharnae was first registered for release in 1991. Sharnae was subsequently withdrawn from commerce in 1998. This review is intended to inform a consideration of the potential for commercialising Sharnae for use in low latitude environments, and to promote awareness of the availability of Sharnae (and other similar germplasms) for future cultivar development or evaluation work in low latitude homoclimates of eastern Australia.

**Key words:** pasture legume(s), plant improvement

Of the wide species diversity in the *Lotus* genus that comprises some 200 species (Kirkbride, 1999), two species (*L. australis*, *L. cruentis*) are endemic to Australia, and four exotic species (*L. angustissimus*, *L. suaveoleus*, *L. corniculatus*, *L. uliginosus*) have naturalised (Wilson, 1980). Of these, only *L. corniculatus* (birdsfoot trefoil) and *L. uliginosus* (greater lotus) are presently in commercial use as pasture plants (Blumenthal and McGraw, 1999).

While the potential zone of adaptation of both birdsfoot trefoil and greater lotus in Australia is substantial (Hill *et al.*, 1996), only greater lotus presently has significant commercial usage (Ayres *et al.*, 2006a; 2006b). Within greater lotus, only two cultivars are commercially available – Grasslands Maku (intraspecific hybrid tetraploid with 25% Mediterranean provenance bred in New Zealand) and Grasslands Sunrise (diploid with 25% Mediterranean provenance bred in New Zealand).

### Greater lotus in Australia

Following commercial release in 1974 of Grasslands Maku, plantings of greater lotus-based pastures in Australia were of the order of 100 hectares per annum during 1978-1983, and increased to 30,000 hectares by 1990 (Harris *et al.*, 1992; 1993). The planted area

subsequently expanded to plateau at about 100,000 hectares by the late 1990's (Blumenthal and McGraw, 1999). The major application of greater lotus has been for beef and dairy cattle in high rainfall coastal districts. Minor applications include groundcover in orchard plantations and seed production (Harris *et al.*, 1993). Greater lotus is currently recommended in New South Wales for cattle and sheep grazing in high rainfall coastal districts and favoured moist sites on the Northern Tablelands (Ayres, 2004).

This relatively low level of usage of greater lotus has evolved through farmer experience with just one cultivar, Grasslands Maku, which has known limitations: Slow establishment and weak seedling vigour especially with cold conditions that accompany autumn planting (Keoghan and Burgess, 1987; Kelman and Bowman, 1996); susceptibility to summer moisture-stress (Blumenthal *et al.*, 1999); poor seed-set at low latitudes (Blumenthal and Harris, 1993); and susceptibility to frost damage (Schiller and Ayres, 1993).

More recently, a state-wide study in New South Wales (Ayres *et al.*, 2006a; 2006b) evaluating the adaptation of greater lotus and birdsfoot trefoil, and allowing for expected adoption levels, proposed that the greater lotus zone in New South Wales could expand to about 1 million hectares, provided that cultivar development achieved broad adaptation to extend the adaptive potential of greater lotus beyond favourable niche environments. Moreover, Ayres *et al.* (2007) proposed that breeding greater lotus for eastern Australia should target the environments most conducive to the warm season growth characteristics of greater lotus. That is, the low latitude summer rainfall region, including the high rainfall coastal zone and medium rainfall tablelands zone in northern New South Wales and south-eastern Queensland. In consequence, appropriate breeding objectives for this environment include better tolerance of summer moisture-stress, stronger seedling vigour and greater seed-set under low latitude conditions.

Species evaluation work in Australia with the *Lotus* genus has previously identified greater lotus as meriting further breeding work (Wilson, 1980; Kelman, 1993). To date, the selection work of Tony O'Brien at Grafton, New South Wales (O'Brien, 1974; 1978) led to the development of the Sharnae germplasm (Wilson, 1992), and the crossing work of Walter Kelman at Canberra, Australian Capital Territory (Kelman and Bowman, 1996) led to the development of the LUX97 germplasm. A recent characterisation study of a working collection of greater lotus cultivars and breeding lines found that these germplasms developed in Australia with high levels of Mediterranean provenance, express high year-round herbage growth, high winter growth activity, and set more seed than Grasslands Maku under low latitude conditions (Ayres *et al.*, 2007).

There is now a considerable knowledge base from research in eastern Australia with the Sharnae germplasm. The present paper reviews this knowledge base to assess Sharnae's potential for cultivar development, or alternatively for its use in future breeding for low latitude homoclimes in Australia and South America. The scientific literature on Sharnae comes mainly from Australian research. A significant number of published documents report on the development, characteristics and agronomic merit of Sharnae. For example: origins (O'Brien, 1978; Wilson, 1980); agronomic merit (Blumenthal *et al.*, 1991; Kelman *et al.*, 1992; Blumenthal *et al.*, 1993; Kelman, 1993; Bowman, 1993; Blumenthal *et al.*,

1994; Blumenthal *et al.*, 1995; Hill *et al.*, 1996; Kelman, 1996; Blumenthal *et al.*, 1999; Blumenthal and McGraw, 1999); seed yield capability (Arango *et al.*, 1998); seed quality (Kelman and Blumenthal, 1992); germination phenomena (Blumenthal *et al.*, 1996); nutritive value (Kelman and Tanner, 1990); and a registration paper provides a “cultivar description” of Sharnae (Wilson, 1992).

Moreover, recent research presents new data on regional adaptation (Ayres *et al.*, 2006a; 2006b), and the expression of morphological characteristics at low latitude (Ayres *et al.*, 2007). Also, a number of unpublished reports held by the senior author provide useful additional resource material. These include a report on the collecting expedition in 1974 that selected the parent germplasm (O’Brien, 1974); a briefing document that considered the case for commercialising Sharnae (Bowman and Wilson, 1992); and miscellaneous memoranda that describe experiences with commercial seed production that led to withdrawal of Sharnae from commerce in 1998 (K.A. Archer, *personal communication*).

## The origins of the Sharnae germplasm

The first public report on the Sharnae germplasm appeared in the *Lotus Newsletter* (O’Brien, 1978). Tony O’Brien, an agronomist with New South Wales Department of Agriculture undertook a *Lotus* collecting expedition in 1974 to the western Mediterranean region (O’Brien, 1974). Amongst other *Lotus* material collected, O’Brien selected three vigorous early flowering greater lotus populations (CPI 67676, CPI 67677, CPI 67678):

*‘I have selected a Lotus pedunculatus type from the Algarve area of Portugal distinct from the L. uliginosus of commerce’* (O’Brien, 1978).

Algarve is located at latitude 37°09’-37°11’ N, the soil at the collection site is of granite derivation, and average annual rainfall (AAR) is 850 mm. It was recorded that “...the site is kept wet by spring-bed soakages” (Wilson, 1992).

A composite of these 3 lines (PI 5303) was evaluated (Wilson, 1980) in north-eastern New South Wales and south-eastern Queensland. PI 5303 performed well but expressed a protracted flowering pattern, which was seen to be a potential problem for seed yield capability. Consequently, selection pressure was applied to CPI 67677 to achieve early and concentrated flowering. Sharnae was developed through long term selection (more than 10 generations) for early and concentrated flowering and high seed production.

## Morphology of Sharnae

Sharnae is diploid,  $2n = 12$  (Grant, 2004). A detailed morphological description is provided by Wilson (1992). Sharnae is a rhizomatous perennial legume that may have erect or decumbent growth habit, depending on the structure of companion vegetation. In comparison with the commercially prominent cultivar Grasslands Maku, Sharnae was noted to be morphologically similar with the exception of being i) less hairy (fewer hairs on the

leaves, calyx and peduncle tip), ii) fewer crown stems and rhizomes, and iii) smaller seed but a greater number of seeds/pod (Wilson, 1992).

*'Sharnae is more robust and bulkier but forms a less dense sward than Maku'* (Wilson, 1992).

Under relatively high latitude (34-35°S) conditions in southern New South Wales, Sharnae (and other Portuguese accessions) expressed greater plant height, equivalent autumn herbage growth but less summer growth, and less rhizomatous spread than Grasslands Maku (Kelman *et al.*, 1997).

In phytotron studies, Sharnae produced fewer, shorter but heavier rhizomes than Grasslands Maku – this was considered to provide an adaptive advantage in environments subject to low rainfall (Blumenthal and Harris, 1998). For environmental conditions where photoperiod ranged from 10-14 hours and temperature was varied diurnally through 15/10, 18/13, 21/16 and 27/22°C (maximum day temperature/minimum night temperature), neither photoperiod nor temperature changed the morphological distinctiveness of Sharnae.

Ayres *et al.* (2007) characterised Sharnae (and a set of other diploid and tetraploid greater lotus populations) under low latitude conditions in northern New South Wales. Vegetative characterisation and herbage yield data were obtained under glasshouse conditions over an extended period. Seed yield components were obtained from field sites at Glen Innes (29°42'S) and Armidale (30°31'S). In comparison with Grasslands Maku, Sharnae expressed larger leaf and thicker stem, greater seed-set, greater herbage yield in all seasons, but relatively weaker early growth vigour.

### **Seed production and seed quality of Sharnae**

At three relatively high latitude sites in southern New South Wales (Nowra: 34°54'S/1166 mm AAR; Moss Vale: 34°28'S/1186 mm AAR; Canberra: 35°18'S/694 mm AAR) where Sharnae and some 38 other greater lotus lines were characterised, Sharnae (and other Portuguese lines) were earlier flowering, set more pods and expressed greater seed yield than Grasslands Maku (Kelman *et al.*, 1997).

Under low latitude conditions (Grafton 29°40'S) in north coastal New South Wales, Sharnae expressed earlier flowering and a more extended flowering pattern than Grasslands Maku (Wilson, 1992). Sharnae commenced flowering in early spring (mid-September) reaching peak flowering by mid-October, and then flowered intermittently until late summer. In this same Grafton environment, Bowman and Wilson (1992) reported seed yield of 314 kg/ha under plot culture. Sharnae expressed double the seed production of Grasslands Maku, smaller seed size, more seeds/pod and 38-45% hard seed content.

Seed yield of 400-800 kg/ha has been achieved in well managed crops grown in favourable seasons (McLaughlin and Clarke, 1989). By comparison, the seed yield of Grasslands Maku in New Zealand averages 250 kg/ha (Blumenthal *et al.*, 1993). At Cobbitty near Sydney

(33°55'S), Arango *et al.* (1998) reported seed yield under irrigated plot culture and intensive insect control of 1.1 tonnes/ha, and found that the combination of low plant density (<15 plants/m<sup>2</sup>) and late harvest (mid-January) maximised seed yield and seed quality. Blumenthal *et al.* (1996) reported 1000 seed weight of 0.743 g for Grasslands Maku compared with 0.532 g for Sharnae. Kelman and Blumenthal (1992) reported a higher level of hardseed in Sharnae (77%) compared with Grasslands Maku (47%) – a feature likely to be advantageous for long-term persistence.

In summary, with sound crop management, seed yield of Sharnae of some 300 kg/ha might be expected. Cultural practices for best seed production include planting in wide rows, keeping the maturing crop moist until 70-80% of pods are ripe, and maintaining vigilant insect control from flowering through pod-set (M.J. Blumenthal, *personal communication*).

### **Germination phenomena of Sharnae**

Under controlled environment conditions, the base temperature for germination of Sharnae is 5°C compared with 6°C for Grasslands Maku (Blumenthal *et al.*, 1996). However, this difference was not considered to provide a major advantage for Sharnae in terms of establishment in cold environments. In this study, Sharnae showed a similar *germination rate*, *hours to first germination* and *hours to 50% germination* compared with Grasslands Maku. Both Sharnae and Grasslands Maku were found to have similarly high threshold temperatures for germination (49.8, 47.3°C, respectively), and the two lines germinated similarly under low moisture conditions.

It is especially noteworthy that the traits *germination rate* and *early flowering* are complementary. Kelman and Forrester (1999) reported that breeding for early flowering (as occurred with Sharnae and LUX97) has i) an indirect positive effect on germination rate and seedling vigour, ii) the expression of these effects is more pronounced in low latitude environments, and iii) germination rate is highly heritable.

### **Agronomic performance**

The greater lotus zone in Australia has been most comprehensively studied in New South Wales. A description of the regions in New South Wales of relevance to greater lotus, and of the agronomic performance of Sharnae in these regions, is provided in the following.

#### ***Humid and sub-humid North Coast (28°11'S- 32°46'S)***

The North Coast region comprises a high rainfall (1200-1600 mm AAR) humid subtropical coastal plain, and a medium rainfall (900-1200 mm AAR) sub-humid hinterland. The rainfall pattern is sharply seasonal with summer dominance, and soils typically are strongly acidic. The major constraints to grazing in this region are the late winter/early spring feed gap (due to erratic spring rainfall) and the low nutritive value of pasture in summer/autumn (associated with the low digestibility of maturing C4 grasses). In this context, the role for greater lotus is to increase the availability of winter/spring herbage and to enhance the quality of autumn pasture for “carry-over” into winter.

In plot trials on two soil types at Grafton (29°40'S/1089 mm AAR), Sharnae produced higher forage yields in late spring and early summer than Grasslands Maku (Bowman, 1993). In grazing trials in the Grafton hinterland, Sharnae persisted better, spread further and produced higher herbage yields than Grasslands Maku on a dry site. Conversely, on a relatively wetter site, Grasslands Maku outperformed Sharnae (Bowman and Wilson, 1992). On a sandy soil at Grafton in combination with Bahia grass (*Paspalum notatum*), Sharnae persisted for four years. In a kikuyu (*Pennisetum clandestinum*) sward at Wollongbar (28°49'S/1700 mm AAR) under dairy cattle grazing, Sharnae persisted for 3 years. However, at Grafton (29°40'S/1089 mm AAR), Sharnae as a companion legume with kikuyu under dairy cattle grazing, disappeared in one year (Bowman and Wilson, 1992). Sharnae outyielded Grasslands Maku at Wingham (31°53'S/1171 mm AAR) in the first year after planting (Bowman and Wilson, 1992).

There is evidence and industry experience that Grasslands Maku does not persist under the episodic drought conditions that prevail in north coastal New South Wales, whereas Sharnae's greater drought tolerance and higher seed production confers survival through adverse dry periods.

#### ***Cool temperate Northern Tablelands (28°55'S - 30°53'S)***

The Northern Tablelands is an elevated (750-1400m) medium rainfall (775-1250mm AAR) landscape where the rainfall pattern is summer dominant, low fertility acidic soils predominate and there is a low photoperiod associated with low latitude and frequent cloud cover. Winters are intensely cold and include some 104 frosts over a 200 day frosting interval. The major stresses for pasture plants are summer moisture-stress, close grazing during winter and episodic drought.

In a grazing study (Ayres *et al.*, 2006a) in which Sharnae was compared with Grasslands Maku under a range of grazing management practices (grazing intensity, seasonal rest, spelling interval, grass competition):-

- a) The botanical presence of Grasslands Maku was markedly seasonal with high presence (ca. 40%) in spring but relatively lower presence (ca. 20%) in winter. Sharnae remained at a low (10-20%) but stable level.
- b) Grass competition affected Sharnae and Grasslands Maku differently. Whereas Grasslands Maku was restricted only by strong grass competition the botanical presence of Sharnae was restricted by both strong and weak grass competition
- c) Sharnae showed a positive response to autumn rest from grazing, especially under lax grazing intensity.

In this cool temperate environment, although the current area of usage of greater lotus is relatively small, greater lotus is considered to be an 'alternative legume' to white clover (*Trifolium repens*). In moist valley-floor landscapes and high altitude/high rainfall niche sites, Grasslands Maku becomes the dominant legume, persists into the long term (>5 years), and enhances the utilisation of low quality grasses (Ayres *et al.*, 2006b). Although there is no significant farmer experience with Sharnae in this region, Sharnae has characteristics that might be expected to confer adaptive advantage and lead to increased usage of greater lotus beyond favourable niche sites presently occupied by Grasslands Maku.

***Temperate South Coast (32°13'S - 37°04'S)***

Rainfall on the South Coast of New South Wales varies from 1,524 mm AAR on the coastal plain and hinterland highlands to 635 mm AAR in inland valleys; most districts receive at least 1,000 mm AAR. Rainfall distribution is slightly seasonal with more rainfall in warm season months than cool season months, and soils are mostly neutral to acidic. Pasture growth is markedly seasonal; low temperature constrains growth in winter and moisture stress limits growth in summer. The role sought for greater lotus in this environment is to provide late summer/autumn feed to offset the decline in growth and quality of white clover-ryegrass pasture (Blumenthal *et al.* 1991, 1993; Blumenthal and Harris, 1991).

In a field characterisation study (Kelman *et al.*, 1992), a collection of 40 accessions of greater lotus were evaluated at two sites (Nowra, 34°54'S/1200 mm AAR; Bowral, 34°28'S/1500 mm AAR). Of these 40 accessions, the New Zealand bred material (Grasslands Maku, G4703, G4704) and a group of Portuguese accessions (including Sharnae) were the most productive. The autumn production of Sharnae was highest at both sites.

***Southern and Monaro Tablelands (34°44'S - 36°30'S)***

In this 'Mediterranean type' climatic zone where AAR is 450-900 mm, winters are moderately cold and soils are typically acidic. Pasture growth is limited by low temperature in winter and hot/dry conditions in summer. The pasture feed-year comprises a flush of growth in spring, sporadic growth in summer-autumn and low growth in winter.

Sharnae outyielded Grasslands Maku at Tumut (35°18' S/907 mm AAR) in the first year after sowing (Bowman and Wilson, 1992). In an evaluation of *Lotus* accessions in the field at Canberra (35°18'S/632 mm AAR) to identify useful germplasm (Kelman, 1996), highlight results included: -

- a) Grasslands Maku was slightly more productive than Sharnae's Portuguese parent (CPI67677) in terms of early vigour and cool season growth, but produced significantly less herbage in summer
- b) CPI 67677 was taller and thicker in the stem, with longer internodes, but was less rhizomatous than Grassland Maku
- c) Unlike Grasslands Maku which produced flower buds but did not set seed, CPI 67677 set seed and recruited seedlings.

Referring to the Portuguese lines, Kelman (1996) said:

*"...while not as productive as New Zealand accessions...these lines are a valuable source of germplasm for breeding cultivars of Lotus pedunculatus which would be better able to set seed over a wide range of latitude in Australia".*

The Portuguese lines CPI 67676, CPI 67677 (Sharnae pre-cursor), CPI 67678 and the New Zealand lines G4703 and G4704 were subsequently crossed (Kelman *et al.*, 1992; Blumenthal *et al.*, 1993; Kelman and Bowman, 1996) to produce BL<sub>polycross</sub> which performance is described in Ayres *et al.* (2007). Although greater lotus breeding work has

occurred at Canberra on the Southern Tablelands, rainfall conditions in this environment are considered to be too marginal for greater lotus to achieve significant commercial usage.

### ***Regional adaptation studies with Sharnae***

1. In a field evaluation (Blumenthal *et al.*, 1991; 1999) of Sharnae, Maku and G4704 at 6 high rainfall coastal sites between latitudes 27°30'S to 38°39'S in eastern Australia:

- a) At no site did Sharnae outperform Maku in terms of plant frequency or herbage mass
- b) Sharnae's performance was limited by poor seedling establishment from which it never recovered. It was considered that the small seed of Sharnae places it at a disadvantage in terms of seedling vigour.
- c) In high latitude temperate sites, where moisture conditions were more favourable and there was less C4 grass competition, Maku had an adaptive advantage provided that summer moisture-stress was not too severe.

2. In a study (Ayres *et al.* 2006 a) in 4 regions in New South Wales (subtropical North Coast, temperate South Coast, cool temperate Northern Tablelands, 'Mediterranean' Southern Tablelands) during a sequence of drought years:-

- a) The effectiveness of nodulation was lower for Sharnae compared with Maku at coastal sites (Casino, 28°53'S/1108 mm AAR, Nowra, 34°54'S/1136 mm AAR), and this was accompanied by a corresponding depression in seedling density
- b) At low latitude sites (Casino, 28°53'S/1108 mm AAR; Glen Innes 29°42'S/849 mm AAR), rhizome growth (rhizome number, rhizome extension) was greater for Maku than Sharnae
- c) There was no seedbank development or seedling recruitment for Sharnae (or Maku) in all 4 regions under study. However, Sharnae declined to low botanical presence at all sites in this sequence of dry years, so floristic expression was not reliably evaluated.

### **Forage quality**

The aspect of forage quality that has received most attention in greater lotus has been the presence of condensed tannins (CT). Because of their protein-binding capacity, CT are associated with bloat protection in ruminants and with increased levels of by-pass protein during rumen digestion. However, high concentrations are also reported to affect palatability and reduce forage intake. In a large collection of greater lotus germplasm, relatively high levels of CT (8 %Dwt) were measured in 6 accessions of greater lotus, including Sharnae (Kelman *et al.*, 1997). In the total pool of accessions from the previous study, a significant negative correlation of CT with forage nitrogen and in vitro dry matter digestibility was found (Kelman 2006). Based on the presence of genetic variation for CT content and herbage productivity among accessions in this collection, a breeding and selection program utilising the Portuguese (Sharnae) accessions resulted in the development of new germplasm (LUX97) with lowered CT content (Kelman *et al.*, 2007).

### **Commercial status of Sharnae**

The Sharnae germplasm was recommended for registration and release by the New South Wales Herbage Plant Liaison Committee in 1991 (Wilson, 1992). An application for Australian Plant Breeder Rights (PBR) as applying under the Plant Breeders Rights Act 1994 was accepted in 1993 and PBR was granted in 1995. However, PBR was surrendered in 1999 following the inability of the licensee to meet the terms of the marketing license. The main problem was the ongoing lack of success by the licensee in producing seed in commercial quantities, which in turn was attributed anecdotally to i) lack of harvesting expertise by contracted farmers with an indeterminate seed crop, ii) difficulties with weed control associated with uncertain herbicide tolerance of Sharnae, and iii) a perception of weak seedling vigour associated with low cold-tolerance.

Sharnae currently has no PBR protection. Accordingly, Sharnae is available i) for merit testing under a germplasm agreement with New South Wales Department of Primary Industries, and ii) for further improvement by crossing or reselection in conjunction with New South Wales Department of Primary Industries, as a pre-requisite to obtaining PBR or its equivalent in other countries and commercialisation. Breeder seed is held by New south Wales Department of Primary Industries at Glen Innes 'Centre for Perennial Grazing Systems'.

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