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Psyllid-resistant *Leucaena* hybrid for northern Australia

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Abstract

Damage caused by the psyllid insect pest (*Heteropsylla cubana*) is the most important limitation to the productivity of *Leucaena leucocephala* ssp. *glabrata* (leucaena) pastures in northern Australia. Psyllid pressure is greatest in humid environments and has restricted the establishment of leucaena pastures to subhumid areas receiving 600-800 mm annual rainfall. Even in subhumid areas, periodic psyllid attack during the growing season can reduce leucaena forage production by 20-50%/year. Plant-based genetic psyllid resistance is present in lesser-known *Leucaena* species. Artificial interspecific hybrids between *L. pallida* (resistant) and *L. leucocephala* are productive and psyllid-resistant but do not breed true-to-type. A 6½-year plant breeding program was undertaken to produce a psyllid-resistant commercial variety. Two cycles of recurrent mass selection and 2 cycles of backcrossing to elite *L. leucocephala* lines have been completed. The program has produced excellent breeding lines ready for stabilization and commercial release. The genetic make-up of these lines is now approximately 87.5% *L. leucocephala* and 12.5% *L. pallida*. They have good bushy tree form for grazing, are vigorous under psyllid attack (over 5x the biomass yield of cv. Tarramba) and have excellent forage quality. Further funding is being sought through MLA's *"Partners in Innovation"* program to finance 3 years of extra work required to complete the stabilization of these lines and the commercialization of the new variety.

A stunted 5-meter long plot of psyllid susceptible *L. leucocephala* ssp. *glabrata* cv. Cunningham in the foreground with a psyllid-resistant backcrossed KX2 hybrid (BC2) in the background. This photograph clearly shows vigorous and productive BC2 breeding lines despite constant heavy psyllid attack 11 months after transplanting.

Executive Summary

Graziers in northern Australia are rapidly adopting leucaena (*Leucaena leucocephala* ssp. *glabrata*) pastures because they are productive, sustainable and profitable. Over 150,000 ha of leucaena have been established in Queensland and this is set to expand to 350,000 ha over the next decade.

Susceptibility to psyllid (*Heteropsylla cubana*) attack is the most important agronomic factor limiting the productivity of existing commercial leucaena varieties. All commercial varieties are defoliated by this pest and plant growth ceases under severe attack. Psyllid pressure is greatest in humid environments, and since its arrival in Australia in 1986, this pest has restricted the establishment of leucaena pastures to subhumid areas with 600-800 mm annual rainfall. Even in these drier environments, periodic psyllid attack during the wet season causes significant (20-50%) production losses.

Plant-based genetic resistance to the psyllid pest exists within the *Leucaena* genus. The University of Queensland (UQ) has been evaluating psyllid-resistance within different *Leucaena* species and interspecific hybrids for 20 years. This work demonstrated the excellent psyllid resistance of *L. pallida*.

In 2002, with support from The Leucaena Network, UQ initiated a 6½ year plant breeding program that aimed to develop a synthetic hybrid variety from superior *L. pallida* × *L. leucocephala* ssp. *glabrata* hybrids bred by the University of Hawaii (UH) through a process of recurrent mass selection. However, after 2 cycles of recurrent mass selection it became apparent that whilst good genetic gains had been made for psyllid resistance, the F_3 generation was experiencing inbreeding depression for yield. In 2005, a change in strategy was adopted in which superior KX2 F_1 and elite KX2 F_3 individuals were backcrossed to elite *L. leucocephala* trees. Two backcross cycles were completed producing breeding lines that are now approximately 87.5% *L. leucocephala* ssp. *glabrata*. The backcross parent used was a superior UH intraspecific *L. leucocephala* ssp. *glabrata* hybrid (K584×cv. Tarramba).

The project has been very successful in achieving its central objective of breeding new material that has excellent psyllid resistance and vigorous biomass yield coupled with the branchy/bushy habit and the excellent forage quality of *L. leucocephala*. UQ now has an array of breeding lines which will lead to the release of a new psyllid resistant cultivar.

There were a number of other positive outcomes from the project:

1. A detailed entomological study into psyllid-host plant interactions was completed which resulted in a peer reviewed scientific publication.
2. A number of undergraduate and postgraduate students conducted their research on aspects of the program (see Appendices 5-7). These enthusiastic young scientists will contribute very significantly to the beef industry in future.
3. Funding support and capacity building of The Leucaena Network has occurred throughout the period of the project.
4. UQ has engaged with The Leucaena Network to effectively transfer information to the beef industry. During the program 23 *Leucaena for Profit & Sustainability* short courses were delivered to >460 graziers. There has been an enormous amount of positive publicity for MLA arising from the project, through numerous articles in Queensland Country Life and other rural press, interviews on public radio and seminars/presentations at industry and scientific conferences and forums (see Appendices 1-4).

However, some components of the final milestone were not achieved. As with all biological research programs, there were several factors beyond our control, that required adjustment of our schedules. These are summarised below:

1. The breeding cycles took longer than expected due to: (a) delayed flowering of desirable plants; (b) poor synchronicity of flowering between pollen donors and elite selected plants; (c) absence of psyllid pressure in 2005 delayed screening of populations; (d) prolonged periods of drizzly rain in 2007 prevented hand-pollination; and (e) bird damage at pollination and of maturing seed in years 2005 & 2007 necessitated the repeat of emasculation and pollination work.
2. The delays described above resulted in each cycle of selection taking over 18 months to complete rather than the 12 months originally predicted.
3. In October/November 2005, a comprehensive data analysis of the F_3 generation indicated inbreeding depression for yield amongst the outcrossed hybrid progeny. In December 2005, new breeding strategies were put in place, but no change was made to the milestone schedules even though some of the criteria for last milestone were no longer relevant. Thus at 30th of June 2008, whilst four cycles of breeding and selection were completed, there was no KX2 F_6 seed produced and it was not possible to complete the following tasks: (a) On-farm producer evaluations and grazing trials for cattle production of F_5 plants; (b) produce 10-20 kg of F_6 seed for bulking to commercial quantities; and (c) PBR preparations underway.

The project was terminated on the 30th of June 2008. A new commercialization phase will be initiated to complete the breeding work and deliver a psyllid-resistant leucaena hybrid to the northern Australian beef industry. However, apart from maintenance of the field site and collection of seed from elite trees, no further work can occur until the new project proposal has been developed and signed.

To complete the breeding process and to make the new variety available to industry, two cycles of progeny testing are required to ensure that the breeding lines do not segregate non-resistant types and breed true-to-type. This process will require an additional 3 years to complete. An additional 12 months will be required to complete a distinctiveness, uniformity & stability trial to gain Australian Plant Breeders Rights (PBR) protection of the variety. A concurrent grazing trial (18-24 months) may be needed to assure graziers of the palatability/acceptability of the new variety to cattle prior to commercial release. However, it is not anticipated that the palatability of the new variety will be compromised as *in vitro* data indicate similar forage quality to existing cultivars.

Commercialization of the variety will be managed by UniQuest, a technology commercialization company owned by UQ, and MLA. UniQuest and MLA are seeking a license partner to purchase the exclusive Australian &/or Worldwide rights to commercialize the new variety. The variety will be protected by PBR held by UniQuest on behalf of UQ and MLA. UniQuest will use the funds raised through the purchase of the license to co-fund the final 3-5 years of its development with matching funding from MLA's *"Partners in Innovation"* program.

Whilst the new variety is not yet available to industry, its future impact will be significant. An additional 1.2 M ha of high-psyllid coastal Queensland will be available for leucaena planting. Furthermore, many graziers located in subhumid Queensland will also adopt the new variety in preference to psyllid susceptible *L. leucocephala* varieties. Utilization of the psyllid-resistant variety will make a significant economic contribution to the northern Australian beef industry by enhancing the supply of high protein forage in grazing enterprises in these environments.

Contents

1 Background

1.1 Importance of leucaena pastures in northern Australia

The rate of adoption of leucaena (*Leucaena leucocephala*)-grass pastures is rising rapidly in northern Australia as graziers realise the extent of the triple-bottom-line benefits. Leucaena is: adapted to clay soils; deep-rooted; drought tolerant; long-lived (>30 years); and tolerant of regular heavy grazing. It efficiently fixes atmospheric nitrogen, requires minimal maintenance, produces forage of exceptional quality (once toxicity has been prevented) and delivers animal performance/carcass quality only slightly inferior to lot feeding. Leucaena pastures are suited to >13 M ha of Queensland, with a current estimated 150,000 ha producing 37,500 tonnes of liveweight gain valued at >$69 M each year. Despite relatively high costs of establishment, this area is expected to expand to 300,000-500,000 ha by 2017 (Shelton & Dalzell 2007). The Queensland Department of Primary Industries and Fisheries (DPI&F) has recognized the ability of leucaena pastures to significantly enhance the productivity/profitability of the beef industry. It has initiated a new *"Accelerated Adoption of Leucaena"* promotion and extension program embedded in the *"CQ Sustainable Farming Systems Project"* to further boost the adoption of leucaena pastures with an aim of getting a further 200,000 ha established in Central Qld in the next 10 years (J. Doughton, personal communication).

1.2 The psyllid insect pest

The leucaena psyllid (*Heteropsylla cubana*) is a small yellow-green insect about 1-2 mm long. It is native to Central America and the Caribbean, where it has presumably co-existed with leucaena for thousands of years. Although it has been reported to occur on a few other leguminous shrubs and trees, these are not damaged to any great extent, and it is probable that the psyllid can only complete its life cycle on plants in the genus *Leucaena* (Bray 1994).

The psyllid first became a problem on experimental plantings in Florida (USA) in 1983. From there it spread rapidly throughout the tropics and sub-tropics and is now present in all areas where leucaena is grown. It is a pantropical pest on leucaena. In Australia, following the first recording at Bowen in north Queensland in April 1986, the insects spread 800 km to Gympie within 3 months, and by mid-October reached Brisbane (Bray 1994). Psyllids are now present wherever leucaena occurs in Australia. The extremely rapid rate of spread suggests that air currents (including high-level winds and cyclone activity) are largely responsible for their dispersal. It is not uncommon to find psyllids on isolated stands of leucaena.

1.2.1 Insect life cycle

Female psyllids lay up to 400 eggs on very young shoots where they are lodged between the folds of the developing leaflets. The eggs are oval, 0.3 mm long and 0.1 mm wide. Newly laid eggs are white, but turn orange or reddish brown after a day or two. Eggs hatch in 2-4 days, and through five nymph stages become adults 10-16 days later, depending on climatic conditions. The nymphs rapidly become mobile and congregate in large numbers on the growing points of young shoots (Bray 1994). Populations can build up extremely rapidly, producing many generations in a year.

In the field, psyllid populations normally fluctuate widely over time. There have been a number of attempts to assess the effect of environmental factors on psyllid population dynamics. Peak numbers

of nymphs tended to occur soon after rain, but are affected by moisture and plant nutrient status, leucaena stand density, humidity and exposure to wind. Management that maximizes forage production (i.e. abundant young leafy growth) greatly increases psyllid numbers.

In Queensland, psyllid numbers are at a maximum during the wet season, whereas in Southeast Asia they are most abundant during the early dry season at the end of monsoon. Psyllid populations are reduced by periods of intense rain (or irrigation), or one or two days of hot (over 35°C) dry winds. Frost will effectively kill psyllids, but also the leucaena.

1.2.2 Damage by psyllids

The leucaena psyllid damages plants when both the nymphs and adults feed by sucking sap from the developing shoots and young foliage. Heavy infestations defoliate the plant and stop growth. Older leaves are not directly damaged by psyllid feeding, but the insects exude drops of sticky fluid causing sooty mould to cover these leaves limiting photosynthesis. Furthermore, sooty mould can grow on adjacent grass covered in honey-dew. The presence of sooty mould can affect the palatability of grass & leucaena, and graziers in Australia and SE Asia report cattle are prone to scouring when feeding on mouldy forage. Where psyllids have been active, there may be no new leaves for a distance of 30-50 cm from the stem tip, representing a loss of up to 10-12 leaves, or several months' growth. Quantification of the damage caused by the psyllid is difficult. In wetter years when psyllids are not controlled, leucaena production in subhumid inland areas can be reduced by 20-50% and up to 40-80% in humid coastal environments (Palmer *et al*. 1989; Bray and Woodroffe 1991).

1.2.3 Options for combating the psyllid

Avoid psyllids
In Australia, most leucaena has been planted in subhumid (600-800 mm annual rainfall) areas where the frequency and severity of psyllid attack is less.

Do nothing
Given time, environmental conditions change and the psyllids disappear. Drought and frosts lead to leaf drop and great reduction in psyllid populations. Very heavy rain (or overhead irrigation) and hot, dry winds will also reduce psyllid populations. However, graziers must be prepared to accept loss of leucaena forage production during these periods of attack.

Spray insecticide
The psyllid is readily killed by low doses of several insecticides. Dimethoate, a systemic insecticide, is registered for use on leucaena and can provide effective control for up to 3-4 weeks (when applied to runoff). An important consideration with the use of insecticides is that beneficial predatory insects will also be killed, and that pesticide residues may be present in the leucaena when fed to animals. Graziers must strictly observe the correct withholding period before grazing or animal sale to ensure pesticide residues are not present in the foliage eaten by livestock and do not contaminate meat. Insecticide use is warranted to protect establishing leucaena, irrigated pastures and high-value seed crops.

Grazing management
Some cattlemen graze their leucaena heavily as soon as the psyllid populations build up in an attempt to remove their feed source and break the population cycle. However, psyllids will generally remain in high numbers and attack new sprouts slowing plant regrowth.

Biological control

In any environment there will be some predators that feed on one or more stages of the life cycle of the psyllid - it has been controlled effectively in its native habitat by natural predators. The larvae of the common ladybird beetles are good predators, but do not seem to be able to keep psyllid populations under control in commercial leucaena pastures. Other useful predators include *Curinus coeruleus* (a beetle that attacks psyllid larvae) and *Psyllaephagus yaseeni,* (a wasp that attacks the eggs of the psyllid) but these have not been released in Australia (Nampopeth in FAO 1994).

The introduction of biological control agents to Australia is a complex procedure because of their possible interactions with other species. For example, one Australian biological control program is seeking to control the tropical weed *Mimosa pigra* through the introduction of a host-specific psyllid of the genus *Heteropsylla*. Introduction of the *Psyllaephagus* wasp to control the leucaena psyllid could negate this program (Bray 1994). There is no current Australian or international effort to source and distribute biological control agents from the psyllids' native range in Mexico & the Caribbean.

Identifying plant-based mechanisms of psyllid resistance

The *Leucaena* genus has been comprehensively investigated for relative resistance to psyllid attack (Mullen *et al*. 2003b), however the biological mechanisms of genetic plant-based psyllid-resistance are not understood. Studies conducted to date have examined factors external to the insect-plant interaction, concentrating on a range of biotic and abiotic factors that affect psyllid populations (e.g. Geiger and Gutierrez 2000). Correlation between tree phenology and psyllid population density has been sought, and the impact of natural enemies has been measured. Population cycles of the psyllid and tree growth cycles were loosely linked. Similarly, populations of natural enemies, whether native or introduced, showed little correlation with the intrinsic rate of increase of psyllid populations and no method for enhancing *Leucaena* productivity could be identified. Emphasis has been placed on pattern rather than process. Understanding the processes and mechanisms of psyllid resistance may facilitate the manipulation of forage production systems to reduce psyllid impact.

Inheritance of resistance appears to be additively dominant with possibly 2-4 genes responsible (J.L. Brewbaker personal communication). There are poor correlations between psyllid resistance and plant forage quality parameters, such as fibre content (NDF, ADF & lignin), total phenolics and condensed tannin concentrations (Castillo *et al*. 1997; Mullen *et al*. 2003b). Detailed fractionation analysis of secondary plant metabolites might reveal unique chemicals with repellent/lethal actions on adult and juvenile psyllids. It appears from entomological studies that resistant species are less attractive to psyllids, which is possibly linked to the production of volatile chemical cues (Finlay-Doney & Walter 2005), and they provide an unsuitable food source for juvenile and adult insects.

Selection and breeding resistant varieties

Despite our limited understanding of the modes of action of psyllid resistance the trait is easily identified based on plant damage ratings in high psyllid pressure environments. An empirical field damage rating scale from 1 (no visible damage) - 9 (death of stem tips) was developed by Wheeler (1988) and this simple scale has proved to be robust and reliable when used extensively all around the world (Mullen *et al*. 2003b,c). Furthermore, this scale has been correlated to yield suppression in a range of *Leucaena* species (Mullen and Shelton 2003). There are three possible approaches to exploiting plant-based psyllid-resistance:

(a) Use of moderately psyllid-tolerant genotypes of *L. leucocephala*. Low levels of tolerance of psyllids exist, with varieties such as K584 and cv. Tarramba showing some ability to grow slowly

under mild to moderate psyllid pressure. These cultivars appear to have long-lived leaves and the capacity to produce many new branches when growing tips are damaged by psyllids. These accessions can provide better growth under mild psyllid attack cf. cvv Cunningham and Peru, however all *L. leucocephala* accessions suffer complete defoliation and stunted growth under sustained heavy psyllid pressure.

(b) Use of other psyllid-resistant *Leucaena* species. A number of lesser known *Leucaena* spp. have genetic plant-based resistance to the psyllid insect pest. Of these, *L. diversifolia* and *L. pallida* are the most promising. There is considerable variation in agronomic traits within these species, and not all lines have the same degree of resistance. However, these species do not appear to be high quality animal feeds having high tannin concentrations, lower palatability & digestibility, and higher plant mortality under repeated defoliation.

(c) Breeding interspecific *Leucaena* hybrids. Hybridisation has been used to improve the agronomic performance of crop species for over a century, but has only recently been applied to tropical agroforestry species. Artificial hybridization aims to produce superior new genotypes by combining the desirable attributes of 2 or more parents. One way of overcoming the psyllid susceptibility of *L. leucocephala* is to use artificial interspecific hybrid breeding programs to introduce psyllid resistance from other lesser known *Leucaena* spp. (Austin *et al*. 1998). There is considerable scope for hybridisation among *Leucaena* species and several naturally occurring hybrids have been reported in the native range (Hughes 1998). The University of Hawaii initiated a hybrid-breeding program with *Leucaena* in the early 1980s and since that time has developed hundreds of inter- and intra-specific crosses. Early research with artificial hybrids concentrated on crosses among the tetraploid accessions, *L. leucocephala*, *L. pallida* and *L. diversifolia*, as these were highly cross-compatible (Sorensson and Brewbaker 1994). There has been very little commercial utilisation of *Leucaena* hybrids due primarily to the high cost of producing F_1 hybrid seed that is produced by laborious hand-pollination. *Leucaena pallida* is a tetraploid species (2n = 104; the same as *L. leucocephala*) that is vigorous, cool tolerant and has low levels of seed production (it is self-incompatible). Artificial interspecific F_1 hybrids between *L. pallida* and *L. leucocephala* (KX2 hybrids) have exhibited good psyllid resistance, exceptionally high biomass yield (the result of heterosis or hybrid vigour) and broad environmental adaptation (Mullen *et al*. 2003a,b,c). They produce palatable forage of good quality that contains condensed tannin concentrations intermediate between both parents (Dalzell & Shelton 2002). Unfortunately these F_1 hybrids cannot be made available for commercial use because F_1 seed cannot be produced on a large scale. Vegetative propagation of these F_1 hybrids has been successful in smallholder farming systems in Southeast Asia (B.F. Mullen personal communication), but this technology is not suited to the broad-acre grazing systems in Australia and South America.

Understanding the mechanisms of psyllid resistance

Research is required to examine the particular interactions (behavioural and physiological) between the pest (psyllid) and its host plant (*Leucaena*), with the primary purpose being to understand how psyllid resistance is conferred. Long distance and contact cues to orient the psyllid to the host, and the effects of plant phenological development on resistance require detailed investigation.

1. ***Long distance cues*** Long distance cues refer to the method by which the psyllid orients to the plant. There is evidence that olfactory, rather than visual cues are more important for the leucaena psyllid (Lapis and Borden 1995). This theory requires testing using a range of susceptible and resistant species. If olfaction is identified as important, the volatile compound(s) responsible need to be identified.

2. ***Contact cues*** Contact cues refer to the processes undertaken by the psyllid to determine the suitability of its host for feeding or oviposition. A preliminary study examined the role of leaf morphological and chemical traits on psyllid damage (Sorensson and Brewbaker 1987). Psyllid damage was poorly correlated with leaf pubescence and mimosine content, while a gum exudate was observed to confer psyllid resistance. More detailed laboratory study investigating the insect activities of palpation, gustation and antennating, is required to understand how plant structures and constituents impact upon these activities.
3. ***Phenological plant development*** There is some evidence that psyllid susceptibility is modified by plant development. For example mature leaf appears to be less susceptible than immature shoots and leaves while the seedlings of some species (e.g. *L. esculenta*) are psyllid susceptible but develop strong resistance with maturity (Sorensson and Brewbaker 1987). The mechanisms for modified susceptibility/resistance require elucidation.

Developing molecular markers for psyllid resistance

Studies of inter- and intra-specific variation in psyllid resistance in the *Leucaena* genus and the aforementioned evaluations of segregating populations of advanced generations of hybrids have revealed the complexity of the genetic basis of psyllid resistance. Correlation between psyllid resistance and many plant morphological, physiological and biochemical attributes has been variable and inconsistent (Castillo 1993; Purdie 1996; Mullen *et al*. 2003b). Significant correlations have been observed between leaf morphological characteristics (rachis and rachilla length, pinna and leaflet number) and chemical characteristics (crude protein, detergent fibre, IVDMD and condensed tannin content). Similarly, F_2 populations of inter-specific hybrids have segregated in a continuum of psyllid resistance between that of both parents (Castillo 1993; Speed and Mullen 2001). These data suggest that psyllid resistance is probably the result of additive effects of multiple gene (polygenic) action. Finding molecular markers for characters regulated by polygenic effects is likely to be difficult. However, the identification of specific modes of psyllid resistance by the entomological approaches described above may expedite the successful identification of molecular markers.

1.3 Production benefits from overcoming the psyllid pest

Benefits to Australia's northern beef industry will be further increased from the greater productivity of a new psyllid-resistant leucaena variety compared to existing varieties, and from the increased area suitable for development to leucaena. Leucaena plantings have primarily occurred in the drier 600-800 mm rainfall regions of central Queensland. Expansion beyond the dry central Queensland environment is currently limited as graziers are unwilling to invest in pasture establishment due to the severe impact of the psyllid damage on leucaena production. A study by Coates (1997) indicated that the psyllid-resistant hybrid would increase the potential range of adaptation of leucaena by 1.24 M ha. This additional area is predominantly in northern Queensland, with smaller areas located in humid coastal areas in central and southern Queensland.

The development of even a limited percentage (0.5% per year) of this additional area to hybrid leucaena systems over 10 years would result in a $39 M increase in gross farm gate returns for northern Australian beef producers. This estimated economic benefit of the psyllid-resistant variety to the beef industry is based on the following conservative assumptions:

(a) pastures of the psyllid-resistant variety yield an additional 100 kg LWG/ha/yr at $1.75/kg compared to pure tropical grass pastures; and

(b) pastures were grazed in the third year after establishment.

Further increases in beef enterprise profitability will accrue from the use of the psyllid-resistant variety in subhumid areas by preventing seasonal forage losses resulting from periodic psyllid infestation during the growing season.

1.4 Other benefits derived from the project

1.4.1 Capacity building within the northern Australian beef industry

An additional benefit was the provision of >$60,000 of financial and advisory support for capacity building of The Leucaena Network. The Leucaena Network was created by the grazier community of central Queensland in 2000 for a specific purpose viz. to tackle the leucaena weed issue and to promote the responsible development of leucaena agroforestry systems. The Network operates under the umbrella of, and links with, existing grazier organisations. It provides leadership, and contributes to research planning and coordination; and training programs for the beef industry. The UQ team provided advice and support to the Network for the duration of the project i.e. from 2002 to 2008 inclusive.

1.4.2 International benefits

The availability of a psyllid-resistant hybrid leucaena will provide multipurpose, economic and environmental benefits to subsistence smallholders, commercial farmers, government agencies and NGOs in developing countries. These vigorous, resilient trees could provide forage, timber and fuel-wood, stabilise landscapes (soil erosion control and dryland salinity amelioration), improve soil fertility and provide shade/support for plantation crops.

2 Project Objectives

The objectives of the project were to:
1. Develop a psyllid-resistant *Leucaena* hybrid for forage production through a 5-year process of recurrent selection (time-frame extended through 2 contract variations to 6.5 years).
2. Identify plant-based mechanisms of psyllid resistance in *Leucaena* (this subprogram was discontinued in 2002).

3 Methodology

3.1 Plant Breeding Program

3.1.1 Recurrent mass selection

From 1 January 2002, the UQ research team was formally contracted to breed a new psyllid resistant leucaena variety by completing 4 cycles of recurrent mass selection in 4 years followed by a year of on-farm trials and seed increase (5 years in total). A vigorous, psyllid resistant hybrid leucaena of superior forage quality was to be bred by recurrent selection. Previous experience (J.L. Brewbaker, personal communication) suggested that 4-6 cycles of recurrent selection were required to achieve a genetically stable hybrid population that breeds true-to-type and would be suitable for commercial release. Each cycle of selection, cross-pollination and seed production was expected to take 12 months. Therefore, 4 cycles of the breeding program was expected to take 5 years depending upon the genetic advances made and the genetic stability of the selected characters in these generations.

A number of UQ undergraduate and postgraduate students contributed to aspects of the plant breeding and evaluation program as part of their research training. Mr Felipe Lemos de Moraes (Graduate Diploma in Agricultural Studies, Brazil) worked on CYCLE 1 (Lemos de Moraes 2002, Appendix 5). Mr Gerardo Solis-Pasos (Master of Philosophy student, Mexico) worked on CYCLE 2 (Solis-Pasos 2005, Appendix 6). Mr Thomas Ryan (4th Year Bachelor of Agricultural Science student, Australia) worked on CYCLE 3 (Ryan 2006).

CYCLE 1 (2001-2003)

The base population for the breeding program comprised F_2 seed collected from 5 superior F_1 parental hybrids (KX2) (Table 1). The F_1 parental hybrids were originally produced by hand-pollination at the University of Hawaii. Open-pollinated seed was collected in June 2001.

Site description

The experiment was conducted at the University of Queensland Research Facility, Redland Bay, Queensland, Australia (27° 37'S, 153° 19'E, altitude 10 m above sea level). The site has a subtropical frost-free climate, receiving 1270 mm of summer dominant (70%) annual rainfall. Average maximum/minimum temperatures are 21/9°C in July and 28/21°C in January. The soil is a fertile, friable and deep krasnozem (oxisol). The land used had a northerly aspect. Psyllid pressure exists year-round, but is often heavier from March-June (Mullen *et al*. 2003b).

Plant material

In reality, the experimental program began prior to 1 January 2002. Seed of the F_2 generation was collected from 10 individual F_1 trees of each of the 5 KX2 hybrids in June 2001. These trees had been established at Redland Bay in September 1993 in agronomic trials evaluating yield, cool tolerance, psyllid resistance, and forage quality (Cuyugan 1995, Castillo *et al*. 1997). Control plants were grown from commercial cv. Tarramba seed (Leucseeds, QLD, Australia) and KX2 F_1 (K748×K636) plants were vegetatively propagated from softwood stem cuttings using the method of Sun *et al*. (1998). During October 2001, seeds of KX2 F_2 and Tarramba were mechanically scarified, surface sterilized (0.1% aqueous sodium dichloroisocyanurate solution), placed on wet paper and incubated at 30°C for 48 hrs. Germinated seedlings were then planted into 40-mm grow tubes containing commercial potting mix in a glasshouse at The University of Queensland, Brisbane (27°28'S, 153°02'E) with average day/night temperatures of 35°C/22°C. After 10 weeks growth, the seedlings were inoculated with commercial *Rhizobium* strain CB3060 and transplanted in the field on the 18-21 December 2001.

Experimental design

A composite F_2 population from 5 randomly outcrossing KX2 F_1 hybrids was evaluated in an α-lattice field trial. Each of the 5 maternal lines was randomly replicated twice between alternate control plants of *L. leucocephala* ssp. *glabrata* cv. Tarramba (K636) and the KX2 F_1 hybrid (*L. pallida* K748× *L. leucocephala* ssp. *glabrata* K636). Trees were planted in rows 2 m apart with 0.5 m between trees within rows. A total of 5382 trees were hand-planted (4892 KX2 F_2 seedlings, 247 cv. Tarramba seedlings and 243 KX2 F_1 rooted cuttings) on an area of approximately 0.5 ha.

Table 1 Open-pollinated KX2 F_1 hybrids used to produce F_2 seed

Parental KX2 F_1 hybrids
L. pallida K806×*L. leucocephala* cv. Tarramba (K636)
L. pallida K748×*L. leucocephala* cv. Tarramba (K636)
×*L. leucocephala* K584
×*L. leucocephala* K658
×*L. leucocephala* K481

Trial management

Six days prior to planting, 40 kg urea/ha (18.4 kg N/ha) and 200 kg/ha of single super phosphate (17.6 kg P/ha, 22 kg S/ha and 40 kg Ca/ha) were applied and mechanically incorporated into the soil. Nitrogen was applied to overcome poor early nodulation and promote rapid establishment. Plants were irrigated when weekly rainfall did not exceed 25 mm. Dead seedlings were replaced regularly during the first 4 weeks after transplanting. Weeds were controlled using pre and post-emergent applications of 400 mL/ha Spinnaker® (240 g/L imazethapyr) (BASF Australia Ltd, Baulkham Hills, NSW, Australia). Post-emergent weeds were also controlled using a mixture of Fusilade® (212 g/L fluazifop-butyl) (Crop Care Australiasia Pty Ltd, Pinkenba, QLD, Australia) 1 L/ha and 1.5 L/ha Basagran® (480 g/L bentazone) (BASF Australia Ltd). Weeds were controlled in the inter-row by rotary hoeing and manual chipping. Hares and kangaroos were excluded from the trial site by a mesh fence.

Measurements and observations

The following measurements were made 127-140 days after transplanting:

(a) Plant height (cm) - from soil level to stem apex.

(b) Basal stem diameter (cm) - within 5 cm of soil level.

(c) Dry matter (DM) production was estimated from plant height (h) and stem diameter (d) using the equation DM index=$h \times d^2$ (Stewart *et al*. 1992). This enabled the non-destructive estimation of biomass yield of the trial plants. Speed (1999) observed a strong correlation (r^2=0.93) between estimated ($h \times d^2$) and actual DM yields for KX2 F_2 plants. Furthermore, these attributes have been shown to be highly correlated across the entire *Leucaena* genus (Mullen and Gutteridge 2002).

(d) Psyllid damage was measured using the psyllid damage rating (PDR) scale of 1-9 of Wheeler (1988) (Table 2).

(e) Tree form was ranked using a rating scale of 1-5 that accounted for the number and degree of primary and secondary branches. A score of 1 indicated the tree was arboreal with little/no branching, while a score of 5 indicated the plant had branched profusely.

(f) Leaf and edible DM production was ranked using a leaf production and retention rating of 1-5, where plants with a rating of 1 had retained few leaves (were very woody) and those with a rating of 5 were extremely leafy.

(g) Degree of flowering was ranked using a rating scale of 1-5 (Table 3).

The sampling period covered 23 days due to persistent rain interruptions between 24 April and 16 May 2002.

Table 2 Psyllid damage rating (PDR) scale developed for *Leucaena* spp. by Wheeler (1988)

Scale	Damage observed
1	No damage observed
2	Slight curling of leaves
3	Tips and leaves curling and yellow
4	Tips and leaves badly curled, yellowish and covered in sap
5	Loss of up to 25% of young leaves
6	Loss of up to 50% of young leaves
7	Loss of up to 75% of young leaves
8	100% loss of leaves and blackening of lower leaves
9	Blackened stem with total leaf loss

Table 3 Rating scale for floral development

Score	Floral development
1	Vegetative growth only
2	Flower buds present
3	Open flowers present
4	Green seed pods present
5	Mature seed pods present

Statistical analysis
The estimated DM BLUEs (best linear unbiased estimators) (see CYCLE 2 Statistical analysis) and the other rank data for the entire F_2 population were interrogated using Microsoft® ACCESS 2000 to identify individual F_2 plants with the following combination of desirable attributes:

(a) estimated DM yield BLUE >mean estimated DM yield BLUE for cv Tarramba;
(b) psyllid damage rating $\leq$2;
(c) branchiness rating $\geq$2.5;
(d) leafiness rating $\geq$2.5; and
(e) floral development rating <3.

This approach was used instead of available hierarchical, agglomerative cluster analysis and ordination analytical software, which could not handle discontinuous or categorical rank data. Histograms and box plots of the rank data were generated in EXCEL (Microsoft® EXCEL 2000) and Minitab 12 (Minitab Inc, PA, USA) respectively.

CYCLE 2 (2003-2005)
The methodology employed was the same as for CYCLE 1 with the following exceptions:

Site description
The experiment was conducted at the Department of Primary Industries and Fisheries (DPI&F), Redlands Research Station, Cleveland, Queensland, Australia (27°53'S, 153° 25' E) as the previous site at Redland Bay was sold by the University of Queensland. The soil at this site is a friable and deep krasnozem (oxisol). The climate is subtropical maritime at 11 m above sea level with an average rainfall of 1278 mm per year. Minimum and maximum temperatures average 20/29°C in January and 8/21°C in August and frosts do not occur.

Plant material
Elite KX2 F_2 individuals were identified from CYCLE 1, based on superior yield, psyllid resistance, leafiness and branchiness, and the remaining inferior trees were killed prior to cross-pollination and F_3 seed production. Unwanted F_2 trees were cut at ground level and re-growth was sprayed with Roundup® (glyphosate, 680 g/kg) (Nufarm Australia Ltd, Laverton North, VIC, Australia) and Brush-Off® (metsulfuron methyl, 600 g/kg) (Dupont (Australia) Ltd, North Sydney, NSW, Australia). A self-compatibility test was applied to all Elite F_2 trees. Three to five inflorescences per tree were covered with glassine bags to prevent pollination from bees. Any self-compatible trees that produced pods from the bagged inflorescences were cut at ground level and eliminated from the population. Only 177 Elite F_2 trees from the original selection of 215 trees were self-incompatible and flowered during the experimental period. Of these, 120 Elite F_2 trees produced seed to form the F_3 generation. Seed was maintained in 120 half-sib families. It was assumed that most of the other 57 flowering F_2 trees had contributed pollen to the F_3 generation. The F_3 seed was collected from March to June 2003.

Bruchid seed beetles (*Acanthoscelides macrophthalmus*) were controlled during seed production by inter-changing applications of two different insecticides, Lorsban 500EC® (chlorpyrifos, 500g/kg) (Dow AgroSciences Australia Ltd, Frenchs Forest, NSW, Australia) and Dimethoate 400® (dimethoate, 400 g/kg) (Farmoz Pty Ltd, Edgecliff, NSW, Australia); both applied at 1 ml/l every 10-14 days.

Seeds of KX2 F_3 and cv. Tarramba were mechanically scarified by cutting a very small piece off the side of the seed coat, surface sterilized (0.1% aqueous sodium dichloroisocyanurate solution), rinsed with water, placed on wet paper with an application of 200 mg/l Banrot® (thiophanate-methyl 250 g/kg, etridiazole 150 g/kg) (Scotts Australia Pty Ltd, Baulkham Hills, NSW, Australia) to control fungi and incubated at 30°C for 48 hrs. Germinated seed was then planted into 40-mm grow tubes containing commercial potting mix in a glasshouse at The University of Queensland, Brisbane (27°28'S, 153°02'E) with average day/night temperatures of 35°C/22°C in June 2003. After 12 weeks growth, seedlings were inoculated with commercial *Rhizobium* strain CB3060 and were transplanted in the field on 1-5 September 2003. Vegetative propagation of the required KX2 F_1 plants had commenced one year earlier using the method of Sun *et al*. (1998), and struck cuttings were maintained in 15-cm pots until the time of planting.

Experimental design

The KX2 F_3 hybrids were evaluated in an α-lattice field trial. F_2 half sib progeny derived from the 5 maternal KX2 F_1 hybrids were randomly replicated twice between diagonal rows of alternate control plants of *L. leucocephala* ssp. *glabrata* cv. Tarramba and the KX2 F_1 hybrid (*L. pallida* K748×*L. leucocephala* ssp. *glabrata* cv. Tarramba). Trees were planted in rows 2 m apart, with 0.5 m between trees within rows. A total of 5722 trees were hand-planted (5239 KX2 F_3 seedlings, 274 cv. Tarramba seedlings and 209 KX2 F_1 rooted cuttings) on an area of approximately 0.8 ha.

Measurements and observations

The same measurements were made (as for CYCLE 1) 301-325 days after transplanting, from 28 June to 23 July 2004. The exception was psyllid damage ratings which were recorded during the period 18-28 February 2004.

Statistical analysis

For all agronomic traits, spatial variation in environmental effects across the plots was accounted for using a linear mixed model by residual maximum likelihood (REML). Environmental variation across the trial site was taken into account by including row and column effects in the linear model, where they were significant. Individual F_2 and F_3 genotypes and the position of these genotypes within rows were fitted as random effects. The fixed effects of the linear mixed model were the overall mean of the F_2 and F_3 populations, and the trends in all traits were derived from the cv. Tarramba repeated check estimated data.

The F_2 and F_3 data for all attributes were initially analysed with genotype as a fixed effect. This provided BLUES for each genotype for each attribute.

Each population was divided in 2 groups for statistical analysis as follows –

F_2	Group 1) progeny of five F_1 parental hybrids Group 2) progeny of unknown parentage*	
F_3	Group 1) progeny of five F_1 parental hybrids Group 2) progeny of unknown parentage*	- progeny were subdivided into 120 half-sib families within F_1 parental hybrids

* plants whose identity (parentage) was accidentally lost during germination and transplanting were designated as "progeny of unknown parentage".

The grouped data were analysed with Group (either 1 or 2) as a fixed term and individuals within group as a random term. For the F_3 data, the random error term within group term was divided into family and individual within family components. These analyses provided variance components for 'between groups' and 'between families within groups' for use in estimating narrow-sense heritabilities for each agronomic trait.

The yield index BLUEs and the other rank data for the entire F_3 population were interrogated using Microsoft® ACCESS 2000 to identify Elite individual F_3 plants with the following combination of desirable attributes:

- Yield index BLUES >600;
- Psyllid damage rating $\leq$2;
- Branchiness >1;
- Leafiness rating >1.

This approach was used instead of available hierarchical, agglomerative cluster analysis and ordination analytical software, which could not handle discontinuous or categorical rank data. Histograms and box plots of the rank data were generated in Microsoft® EXCEL 2000 and Minitab 12 respectively.

Inbreeding coefficients (F) for each cycle of selection were determined from the pedigree structure of the breeding program, based on the assumption that there was no self-pollination, no pollen contamination from outside the KX2 populations and equal pollen contribution from all parents. Genetic recombination of the tetraploid KX2 trees was assumed to behave like diploids, because both parent species are allotetraploids (Hughes 1998). Therefore KX2 hybrids are likely to contain 2 non-complementary genomes, with little recombination occurring in sets of homologous chromosomes (M.J. Dieters personal communication). Inbreeding coefficients, or degree of relationship between parents, of 0.117 and 0.271 were calculated for the F_2 and F_3 generations respectively, and were similar to those expected in half-sib and full-sib matings (F=0.125 and 0.25 respectively) as the 5 elite F_1 hybrid parents were closely related. Whilst this experiment was not designed to specifically investigate the heritability of the agronomic traits, estimates of narrow-sense heritability were made. The inbreeding coefficients were used to calculate a multiplier of 1.845 for the 'between family variance' in the F_2 population to enable the estimation of heritability for each trait from the F_3 population. The partitioned variance components from the ANOVA of F_3 data were then used to estimate heritability.

3.1.2 Backcrossing program

The lower than expected biomass yield data was discussed with plant breeders at The University of Queensland. We concluded that the yield data and pattern of segregation within the F_3 population indicated inbreeding depression. This usually occurs when related individuals, that have hidden (recessive) deleterious genes, are mated together. It is often a problem in hybrid populations that exhibit heterosis, as with this interspecific KX2 hybrid. Elite parents have superior agronomic performance, however when open-pollinated (out-crossed), a large proportion of their progeny are homozygous for the deleterious genes and therefore perform poorly. Biomass yield could be expected to decline further in future advanced generations of the KX2 hybrid if the current open-pollinated breeding program were pursued unchanged.

Fortunately, two robust alternative breeding strategies were available to capitalize on the genetic progress made to date in fixing psyllid-resistance in the elite F_3 individuals. They were:

1. In CYCLE 3 backcrossed lines would be developed by backcrossing elite *L. leucocephala* ssp. *glabrata* genotypes (e.g. an intraspecific *L. leucocephala* ssp. *glabrata* hybrid) to KX2 F_1 and the most psyllid-resistant elite KX2 F_3 trees. Backcross progeny were screened for psyllid resistance and the resistant individuals backcrossed again in CYCLE 4. The end product of this strategy would be a leucaena variety genetically identical to *L leucocephala* ssp. *glabrata* but with psyllid resistance from the KX2 hybrid.
2. In CYCLE 3, inbred lines of advanced generation self-compatible KX2 hybrids would be developed. The objective of this strategy was to 'fix' the desirable genes of these elite trees into homozygous lines of adequate genetic uniformity. It was anticipated that genetic stability would be achieved more quickly using an inbreeding approach (self-fertilization) compared to the original out-crossing strategy. The end product of this strategy would be a single inbred line (variety) or combination of inbred lines to create a synthetic variety.

Due to the unforeseen problem of inbreeding depression, delays in the experimental program occurred while the alternative breeding strategies were discussed and developed. An extensive program of self-fertilization and manual backcrossing was undertaken from February 2005.

The research team had to acquire the necessary technical skills to carry out the backcrossing program. This involved study of the phenological development of flowering biology to predict rates of flower bud development and anthesis; identification and control of insect pests that damaged leucaena flower buds; techniques for the emasculation of flowers, collection of pollen and hand pollination of flowers (developed by Dr Charles Sorensson at the University of Hawaii) (Plates 1-3). The emasculations were performed between 12 midnight and 5am in the morning. Developing a seed orchard management protocol and understanding flower development resulted in successful artificial hybridization of *Leucaena* being routinely achieved.

An elite UH intraspecific *L. leucocephala* ssp. *glabrata* hybrid (K584×cv. Tarramba) was backcrossed twice to the F_1 parent hybrid *L. pallida* K748×cv. Tarramba and the most psyllid-resistant of the elite F_3 hybrid trees. Backcross progeny were screened for psyllid resistance and the resistant individuals backcrossed again.

CYCLE 3 (2005-2007)

The methodology employed was the same as for CYCLE 1 with the following exceptions:

Site description

As for CYCLE 2.

Plant material

The breeding lines evaluated in CYCLE 3 originated from 4 different sources:

(a) Outcrossed F_4 seed collected from open pollinated elite F_3 plants identified in CYCLE 2;

(b) Selfed F_4 seed collected from elite F_3 plants identified in CYCLE 2;

(c) F_3 BC1 seed produced by manually emasculating flowers of elite F3 plants (performed between 12 midnight and 5am) identified in CYCLE 2 and hand pollinating these flowers with pollen collected from K584×cv. Tarramba hybrids using the method of Sorensson (1988); and

(d) F_1 BC1 seed produced by manually emasculating flowers of the F_1 hybrid *L .pallida* K748×cv. Tarramba and hand pollinating these flowers with pollen collected from K584×cv. Tarramba hybrids.

Seedlings were inoculated with *Rhizobium* strain CB3126 and transplanted in the field on 6-7 December 2005.

Experimental design
A row column design was used that contained 78 breeding lines arranged in 6 blocks (replicates). Each replicate comprised 6 rows 2 m apart and contained 13 five metre contiguous plots. Each plot contained 10 plants sown 0.5 m apart. Rows of highly psyllid-susceptible cv. Peru were planted as borders around the edge of the trial and every 3rd row throughout the trial to ensure the even distribution of psyllid pressure.

Measurements and observations
The same measurements were made (as for CYCLE 1) 118-127 days after transplanting, from 3-12 April 2006.

Statistical analysis
The data were analysed by ANOVA in a row-column design (SAS v9, SAS Institute Inc, NC, USA). The analyses incorporated multiple comparisons between the variance components of breeding lines, rows, columns and reps for the PDR and YI data. These analyses permitted the comparison of relative performance amongst breeding lines and the comparison of individual breeding lines to the control commercial variety cv. Cunningham. Additional analysis enabled the comparison between the average performance of breeding lines grouped as F_1 BC1, F_3 BC1, F_3 selfed lines & F_4 OC lines. Within superior breeding lines, elite individual plants were selected that were vigorous, psyllid-resistant (PDR<3) and with good tree form and leaf retention.

CYCLE 4 (2007-2008)
The methodology employed was the same as for CYCLE 3 with the following exceptions:

Plant material
Elite BC1 plants identified in CYCLE 3 were manually emasculated and hand-pollinated with pollen collected from K584×cv.Tarramba hybrids as described for CYCLE 3. All seedlings were transplanted into the field on 26-27th of November 2007.

Experimental design
Only 4 replicates of each block were established.

Measurements and observations
The same measurements were made (as described for CYCLE 1) 127-136 days after transplanting, from 3 to 11 April 2008.

Statistical analysis
The data were analysed by ANOVA in a row-column design (SAS v9). The analyses incorporated multiple comparisons between the variance components of breeding lines, rows, columns and reps for the PDR and YI data. These analyses permitted the comparison of relative performance amongst breeding lines and the comparison of individual breeding lines to the control commercial variety cv.

Cunningham. Additional analysis enabled the comparison between the average performance of breeding lines grouped as F_1 BC2, F_3 BC2, selfed lines & F_5 OC lines.

3.2 Forage Quality Assessment

This component of the research program was undertaken by Miss Emily Litzow for her 4th year research project as part of her UQ Bachelor of Agricultural Science degree (see Appendix 7). Agronomically elite plants were selected from CYCLE 3 and included progeny of backcrosses (BC) from both F_1 and F_3 hybrids, outcrosses (OC) and trees of commercial *L. leucocephala* ssp. *glabrata* varieties cvv Tarramba (T) and Cunningham (C).

Sampling procedure
The sampling procedure was carried out in February 2007. Youngest fully expanded leaf samples were taken from 70 individual *Leucaena* plants, representing 13 F_1 BC, 16 F_3 BC, 21 OC, 10 C & 10 T plants. The samples were then field frozen on pelleted dry ice (-78°C) to preserve the condensed tannins in an *in vivo* state (Dalzell and Shelton 1997). The leaves were then freeze dried and ground to pass a 1-mm sieve.

Crude protein
Crude protein was estimated by determining the N concentration in the leaves using combustion analysis (LECO® CNS 2000, LECO Corporation, St. Joseph, MI, USA). Crude protein content was then calculated using the equation CP (% DM) = N content (% DM)×6.25.

***In vitro* dry matter digestibility**
In vitro DM digestibility (IVDMD) represents the proportion of a feed that is capable of being digested by animal or microbial enzymes. The assessment method mimics the digestive processes that occur in a ruminant's digestive tract, but it is performed in a laboratory using enzymes. The IVDMD of the leaf samples was estimated using the pepsin-cellulase digestion technique of McLeod and Minson (1978).

Condensed tannins
The method used for extractable tannin analysis the modified proanthocyanidin (butanol/HCl) assay of Dalzell and Kerven (1998). The proanthocyanidin (butanol/HCl) method of Dalzell and Kerven (2002) was used to measure total bound (protein- and fibre-bound) condensed tannins.

Statistical analysis
One-way ANOVA using Dunnett's mean comparisons (Minitab 15, Minitab Inc, PA, USA) were conducted to test if the breeding lines differed from commercial varieties for each forage quality parameter. Scatter plots and linear regressions were used to determine relationships between forage quality parameters.

3.3 Entomological Research Program

3.3.1 Identification of plant-based mechanisms of psyllid-resistance

Ms Mary Finlay-Doney undertook the first phase of the entomological research program under the supervision of A/Prof Gimme Walter, Entomology, UQ as her Honours research program for a UQ

Bachelor of Science degree. This work has been published in the journal *Agricultural and Forestry Entomology* (Finlay-Doney and Walter 2005)(see Appendix 4) and key findings are summarized here.

The following hypotheses were tested:
1) Long-distance prealightment cues lead to differential arrival rates on flushing growth (actively growing shoots and young leaves) of *Leucaena* species.
2) These long-distance cues may be visual and olfactory in nature.
3) Short distance host differentiation is related to chemical and physical properties of the plant surface.
4) Different *Leucaena* spp. will differ in suitability as hosts for oviposition and nymphal instars.

Field adult psyllid counts
Field trials to measure psyllid invasion rates were conducted at UQ's Redland Bay Research Facility, where potted plants of *L. leucocephala* cv. Tarramba, F_1 hybrid *L. pallida* K748×*L. leucocephala* cv. Tarramba and *L. pallida* were placed amongst established hedgerows of leucaena infested by the psyllid. Numbers of adult psyllids were counted on plants in numerous replicated runs over different seasons and different psyllid pressure conditions (from mild to heavy).

Field egg and immature psyllid counts
Samples of young growing points were collected from 12 plants randomly selected from 1 ha monoculture plantings of the 3 *Leucaena* species growing at UQ's Mt Cotton Research Facility. The number of eggs, nymphs and adults present were counted or scored.

Assessing visual cues by reflectance tests
Wavelength reflectance spectra (brightness and chroma) were recorded for the 3 *Leucaena* species using a flash spectrophotometer between 300-700 nm. Readings were compared to a white barium sulphate standard and were taken from both the growing points (were psyllids congregate) and from mature leaves for 12 individual plants from the 3 species.

Assessing olfactory cues by headspace volatile analysis
Gas was collected from the growing points of the 3 *Leucaena* species that were enclosed in plastic bags between March and May 2002. Volatile chemicals released by the plants were captured in special filters and then eluted and stored in dichloromethane prior to analysis by gas chromatography. Nine to 12 individual plants were tested for each species.

Caryophyllene field tests
Psyllid traps (green plastic card) were baited with paraffin oil containing 6 concentrations (0-10% v/v) of ß-caryophyllene ((–)-trans-caryophyllene, CAS number 87-44-5, product C-9653). Traps were located at the Redland Bay Research Facility surrounding a mixed plantation of *Leucaena* species that was infested with psyllids. Three replicates of each trap were placed in the field for 24 hrs over 4 consecutive days.

Short range host selection
Short range host discrimination trials were conducting in the laboratory on artificially reared adult psyllids. Individual insects were exposed to the growing points of the 3 *Leucaena* species spaced equidistantly in plastic containers. Sex of the psyllid and the first growing point contacted were recorded.

4 Results and Discussion

4.1 Plant Breeding Program

4.1.1 Recurrent mass selection

CYCLE 1 (2001-2003)

The KX2 F_2 population segregated widely for all agronomic traits of interest (Table 4) confirming previous findings of Castillo *et al*. (1994) and Wheeler *et al*. (1994) who worked with a KX2 F_2 population based on different accessions (*L. pallida* K376×*L. leucocephala* K8). This contrasted with uniformity of traits exhibited by KX2 F_1 hybrids observed in this and other studies (Sorensson 1995).

Average biomass yield of the entire KX2 F_2 population was lower than that of cv. Tarramba and less than half that of the KX2 F_1 plants. The poorer average F_2 population performance and the production of poor unthrifty plants were attributed to the presence of inferior recessive alleles inherited from *L. pallida*. Due to self-sterility, *L. pallida* appears to be highly heterozygous for many important yield determining genes/loci. The recessive alleles are hidden in the vigorous *L. pallida* parents and within the KX2 F_1 hybrids. They only become apparent once they recombine in homozygous combinations in inferior F_2 plants. These inferior genes can be eliminated from a plant population through repetitive intense selection, thereby accumulating superior alleles for important genes/loci in homozygous elite individuals. Of the 5 KX2 F_1 parental lines, progeny of K748×K636 were the most vigorous.

Psyllid damage ratings (PDR) were very high for cv. Tarramba, with plants exhibiting 25-50% defoliation of the growing point. This indicated psyllid pressure was high enough to enable the selection of resistant plants. In contrast, on average, all the KX2 lines were highly psyllid resistant (average ratings 1.5-2.6). However, in each of the segregating F_2 populations a small proportion of individuals was highly susceptible (PDR>5).

It was possible to select elite F_2 individuals that were high yielding, psyllid-resistant, with good tree form and high levels of leaf production and retention. A population of 412 individuals was selected to be parents for the next cycle of breeding. This population had an average yield 90% higher than cv. Tarramba and an average PDR of a mere 1.4.

CYCLE 2 (2003-2004)

The KX2 F_3 population segregated again for all traits of interest. What was not expected was the comparatively low average performance of the entire KX2 F_3 population, which yielded only 27% that of cv. Tarramba (Table 5). By comparison, the entire KX2 F_2 population yielded 79% that of cv. Tarramba. More detailed analysis of the performance of the KX2 F_3 population revealed disproportionately high numbers (a skewed distribution) of very low yielding plants. This occurred despite applying intense selection pressure for the yield trait to the KX2 F_2 population, where the average yield of the Elite KX2 F_2 parents was 190% that of cv. Tarramba. This population distribution and the poor overall performance of the KX2 F_3 population indicated that the outcrossing recurrent mass selection breeding program was suffering inbreeding depression for yield. This was not expected or previously experienced in similar work in Hawaii (J.L. Brewbaker, personal communication). As previously discussed, this result led to a change in breeding strategy in CYCLES 3 and 4 to a backcrossing program.

Table 4. Mean biomass yield index (± standard error) of different *Leucaena* breeding populations 127-140 days after transplanting in CYCLE 1 (2001/2003).

Population	Yield Index (cm^3)	Psyllid Damage Rating (PDR) (scale 0-9)[#]	Number of plants (n)
Tarramba	2741±62 **c**[*]	5.7±0.09 **a**[*]	247
KX2 F_1	7528±544 **a**	1.8±0.03 **b**	27
Entire KX2 F_2	2301±31 **d**	2.2±0.02 **b**	4873
Elite KX2 F_2	5232±114 **b**	1.4±0.02 **c**	412
Parental KX2 F_1 lines			
K806×cv. Tarramba F_2	1683±75	2.4±0.09	559
K748×cv. Tarramba F_2	3695±103	1.6±0.04	678
K748×K481 F_2	2035±58	2.3±0.04	1236
K748×K584 F_2	1964±51	2.6±0.05	1221
K748×K658 F_2	2417±61	2.1±0.03	1177

Scale of Wheeler (1988); 0 = no damage through to 9 = stem death.
* Means within columns followed by different letter are significantly different (P<0.05) derived from BLUE data.

Table 5. Mean biomass yield index (± standard error) of different *Leucaena* breeding populations 301-325 days after transplanting in CYCLE 2 (2003/2004).

Population	Yield Index (cm^3)	Psyllid Damage Rating (PDR) (scale 0-9)[#]	Number of plants (n)
Tarramba	16033±320 **a**[*]	6.1±0.07 **a**[*]	274
KX2 F_1	15215±382 **a**	3.2±0.07 **b**	210
Entire KX2 F_3	4347±65 **c**	2.4±0.02 **c**	5242
Elite Kx2 F_3	13488±201 **b**	1.6±0.02 **d**	460

Scale of Wheeler (1988); 0 = no damage through to 9 = stem death.
* Means within columns followed by different letter are significantly different (P<0.05) derived from BLUE data.

Periods of severe psyllid attack occurred resulting greater psyllid pressure in CYCLE 2 compared with CYCLE 1, with cv. Tarramba having a mean PDR value over 6 indicating >50% of young leaflets had been defoliated from the growing points. This level of damage equates to 100% DM yield suppression (i.e. the cv. Tarramba plants stopped growing). Psyllid pressure was exerted uniformly across the trial site enabling confident selection of resistant individual plants. Like the KX2 F_2 population, the F_3 population segregated widely for resistance. However, average resistance within the F_3 population remained low and similar to that of the F_2 population, indicating that genetic gains in resistance made in the recurrent selection breeding program were reasonably stable (Table 5).

Heritability of traits

Assuming no dominance effects, narrow-sense heritability was calculated from inbreeding coefficients and variance components from ANOVA for each trait. Heritability of yield from the F_2 to the F_3 population was 0.210±0.037 (but negative - i.e. depressed yield), psyllid-resistance 0.194±0.028, branchiness 0.127±0.022 and leafiness 0.177±0.028. Whilst this study was not specifically designed to measure heritability of the agronomic traits of interest, the heritability estimates made in this study were similar to those reported elsewhere for yield (Austin 1995) and psyllid-resistance (Austin *et al*. 1995; Pan 2000) in *Leucaena*.

CYCLE 3 (2005-2006)

Progeny of the 4 revised breeding strategies were evaluated. The backcrossed lines (progeny from backcross cycle 1 = BC1) generated from superior F_1 and F_3 KX2 parents had excellent agronomic performance. Theoretically, the genetic makeup of these lines was 25% *L. pallida* and 75% *L. leucocephala*. The BC1 progeny had vigorous and consistent growth indicating that the adoption of this breeding strategy had begun to overcome the inbreeding depression problem (Table 6). Furthermore, these lines had an average PDR of 3 to 4 that was much lower than the *L. leucocephala* reference varieties (PDR=5-7), even though these BC1 plants were heterozygous (i.e. had both susceptible and resistance alleles) for psyllid resistance. Within these BC1 breeding lines, superior individuals that out-yielded cv. Cunningham by over 200% and had high levels of resistance (PDR less than 3) were identified and selected for further backcrossing (Table 6). All BC1 plants had good tree form with a high degree of basal and secondary branching that should ensure that forage produced will remain within reach of grazing animals. The research team was confident that the backcross strategy would deliver a superior psyllid-resistant *L. leucocephala*-type variety to the northern Australian beef industry.

Unfortunately, the inbreeding (self-pollinated) strategy failed to deliver any superior KX2 breeding lines. All the F_3 selfed lines developed had lower biomass yield than cv. Cunningham (Table 6), despite having good levels of psyllid-resistance (average PDR=2). This result confirmed the presence of large numbers of recessive deleterious genes/gene complexes within the elite KX2 F_3 plants. This breeding strategy was discontinued.

The original recurrent selection outcrossed (cross pollinated) strategy was continued on a reduced scale. The F_4 population continued to exhibit excellent levels of psyllid resistance but segregated widely for all other agronomic traits of interest. This indicated that many more cycles of intense selection would be required to stabilize an elite line of KX2 hybrids and eliminate the deleterious 'junk' genes that were responsible for creating inbreeding depression for yield. Despite this segregation, elite plants that were vigorous and psyllid-resistant were identified. Out of academic interest, a small number of these elite KX2 F_4 trees were allowed to outcross again and F_5 seed was collected for evaluation in CYCLE 4.

Table 6. Mean biomass yield index (± standard error) of different *Leucaena* breeding populations 118-127 days after transplanting in CYCLE 3 (2005/2006).

Reference lines	Yield Index (cm^3)	Psyllid Damage Rating (PDR) (scale 0-9)[#]	Number of plants (n)
Peru	3779±239	7.3±0.09	60
Cunningham	4180±162	7.2±0.07	89
Tarramba	*	5.8±0.08	188
L×L BC parent	5119±125	5.2±0.08	121
L. pallida (K748)	4169±265	1.7±0.14	69
Mean of breeding strategies			
F_1 BC1 lines	4709±193	3.8±0.12	475
F_3 BC1 lines	5109±141	2.9±0.09	944
F_3 selfed lines	1821±202	2.1±0.12	538
F_3 outcrossed lines	3984±122	1.9±0.08	1664
Mean of elite individuals selected as parents for CYCLE 4			
F_1 BC1 lines	9449±336 (212%)^	2.3±0.11	25
F_3 BC1 lines	11187±321 (251%)	2.3±0.13	33
F_3 outcrossed lines	11973±285 (269%)	1.6±0.10	31

[#] Scale of Wheeler (1988); 0 = no damage through to 9 = stem death.
* Tarramba was planted 3 weeks later than other plants so Yield Index not presented for comparison.
^ Yield relative to the average performance of cv. Cunningham is presented in parenthesis.

CYCLE 4 (2007-2008)

Backcross and outcrossed progeny were evaluated under continuously intense psyllid pressure at the Cleveland site during December 2007 to April 2008. As a result of this prolonged period of psyllid infestation and damage the yield of the susceptible commercial *L. leucocephala* ssp. *glabrata* varieties was very poor (Table 7). By contrast, the yield of the psyllid-resistant BC2 progeny and the outcrossed KX2 F_5 plants was much higher. These conditions were ideal for selecting individual plants for psyllid resistance. Psyllid damage ratings across all varieties and breeding lines were higher in CYCLE 4 compared to CYCLE 3. Five elite BC2 lines (2 originating from F_1 BC1 and 3 from F_3 BC1 plants) were identified that had consistently good biomass yield, psyllid resistance and tree form (Table 7). The best 10 individual trees of each of these breeding lines (50 in total) were selected for progeny testing. An additional 30 individual trees were also selected from another 14 BC2 lines for progeny testing and evaluation. Proximate forage quality analysis of leaves of these trees will be completed in other UQ work. These 80 trees will be self-pollinated and the selfed seed grown out for 2 additional cycles of evaluation to determine their genetic stability and suitability for commercial release (see 'FUTURE WORK' below).

The F_5 outcrossed progeny exhibited different levels of uniformity. Most were still highly variable and segregated widely for all agronomic traits of interest. However, 3 lines were reasonably uniform, very productive and had very high levels of psyllid resistance. Unfortunately they are likely to have inferior

forage quality (similar to *L. pallida*) and may also be less tolerant of regular defoliation or grazing, however they have potential for use as biomass/bioenergy crops or multipurpose trees in subsistence agriculture.

Table 7. Mean biomass yield index (± standard error) of different *Leucaena* breeding populations 127-136 days after transplanting in CYCLE 4 (2007/2008).

Reference lines	Yield Index (cm^3)	Psyllid Damage Rating (PDR) (scale 0-9)#	Number of plants (n)
Peru	214±67	7.3±0.05	40
Cunningham	147±47	7.1±0.07	40
Tarramba	386±73	6.8±0.03	40
L×L BC parent	412±89	6.5±0.04	42
L. pallida (K748)	2160±193	1.0±0.00	27
Best BC2 lines			
F_1 BC2 line A	1837±205	3.4±0.03	60
F_1 BC2 line B	1846±165	3.1±0.02	40
F_3 BC2 line A	1405±303	2.6±0.03	38
F_3 BC2 line B	1489±199	2.6±0.02	39
F_3 BC2 line C	1526±228	3.4±0.04	54
Mean of elite BC individuals selected as parents			
From 5 best BC2 lines	3161±92	2.4±0.08	50
From 14 other BC2 lines	3483±94	2.4±0.15	30
Best OC lines			
OC 17	2844±240	1.1±0.01	40
OC 10	2806±385	1.5±0.01	40

Scale of Wheeler (1988); 0 = no damage through to 9 = stem death.

FUTURE WORK

Further work is required to: stabilize the best backcross breeding lines; successfully gain Australian Plant Breeder's Rights (PBR) protection of the new variety; and conduct a preliminary grazing trial. The following work needs to be completed prior to the commercial release of the new variety to industry for seed production and retail sale.

Activity 1. Additional progeny testing to stabilize the best backcross breeding lines

Two additional cycles of progeny testing are required to complete the development of the new variety prior to delivery to the market place. This extra work is needed to stabilize the genetic uniformity of the elite BC2 lines. Progeny from 1 of these elite lines will be selected for release as the new variety. This process is expected to require 2 generations and to take 3 years.

The first cycle of selection is required to identify individuals that are homozygous for psyllid resistance. The self-fertilized seed from BC2 plants will segregate for psyllid resistance as described in the simple genetic analyse diagram below where R represents dominant resistance alleles and r represents recessive susceptible alleles for the hypothetical single gene for psyllid resistance (& additive dominance of alleles):

BC2 × BC2
(Rr) (Rr)
↓
RR Rr Rr rr

Therefore we expect that about 25% of progeny will be highly susceptible to the psyllid (rr) while 50% have intermediate resistance (Rr) and 25% are highly psyllid-resistant (RR). In reality we believe there are 2-4 genes responsible for conferring psyllid-resistance however they do appear to act in the same simple way. The fact that more genes are involved increases & complicates the frequency distribution of genetic combinations and therefore degrees of psyllid resistance expressed. This results in a lower proportion (probably <10%) of progeny being homozygous for psyllid resistance. The homozygous, highly psyllid-resistant plants will be selected and then self-fertilized and seed collected.

The second cycle of evaluation will involve testing of the progeny generated from the self-fertilized elite plants identified in the previous trial. The primary objective of the progeny test is to prove that these elite plants are genetically stable (i.e. all progeny exhibit a uniform high degree of psyllid resistance). Once stability has been proved, seed will be collected from the 100 progeny tested and the best line will be chosen. In this way the progeny test could act as a seed orchard to bulk seed supplies ready for release to the licensee(s) contracted to produce and market the new variety. This will bring to completion the plant breeding work.

Activity 2. Apply for PBR protection for new variety

A 'DUS' trial will be required to gain PBR for the new variety. This trial is required to prove that:

1. the new variety is **D**istinct from existing varieties;
2. it is **U**niform (plants are similar within a generation); and
3. that it is genetically **S**table across generations.

The trial will involve comparing the agronomic performance of the new variety with cvv Peru, Cunningham and Tarramba in a high psyllid-challenge environment. It will also require comparing 2 generations of the variety (generated from the progeny testing cycles) to test for genetic stability across generations. This process must be overseen by a Qualified Person (QP) registered with the Australian PBR Office. This process will take 12 months to complete.

Activity 3. Conduct a grazing trial to determine the palatability and consumption of the new variety

It is anticipated that the new variety will have the same excellent forage quality and palatability attributes of *L. leucocephala* ssp. *glabrata*. Laboratory proximate analyses have been undertaken throughout the breeding program (see below) and indicate the key forage quality parameters, crude protein content, *in vitro* dry matter digestibility and tannin concentration are similar in the new hybrid variety and existing commercial varieties. However, *Leucaena pallida* has proved to be an inferior forage with low and variable palatability, poor digestibility and has delivered inferior animal liveweight gain (Jones *et al*. 1998). Given the new variety will still contain 12.5% *L. pallida* genes; it would be prudent to undertake a short-term (2 years) grazing trial.

4.2 Forage Quality Assessment of 1st Backcross Cycle

4.2.1 Crude protein

Crude protein content in the YFEL of the commercial varieties cvv Cunningham and Tarramba ranged from 22.5-25.5% DM. Both the BC1 and OC breeding lines had similar crude protein contents to these varieties ranging from 22-25.5%. This indicated that the nitrogen fixing ability of the new breeding lines was the same as that of existing commercial varieties. Therefore, these breeding lines should produce forage legumes high in crude protein to supplement tropical grass pastures.

4.2.2 *In* vitro dry matter digestibility

This trait was more variable among individual plants of commercial varieties and breeding lines than crude protein content. Greatest variability was observed in the OC breeding lines (range 50-58% DM) followed by F_3 BC1 lines (range 50-60% DM) and reflected the higher and variable genetic contribution of *L. pallida*. The F_1 BC1 (mean 57% DM) and cv. Cunningham (mean 61% DM) had IVDMD levels higher ($P<0.05$) than cv. Tarramba (mean 54% DM), while F_3 BC1 (mean 57% DM) and OC lines (mean 54% DM) were not significantly different from cv. Tarramba. Cunningham had significantly higher IVDMD than all other lines ($P<0.05$). Thus the average IVDMD of all the breeding lines was ranked between cv. Tarramba and cv. Cunningham.

4.2.3 Condensed tannins

Extractable (ECT) and bound (BCT) tannins were both assessed and analysed as well as the total tannin (TCT) content of the plants.

Extractable condensed tannin content
The OC breeding lines had the highest (mean 18% DM) and most variable (range 8-35% DM) ECT content. This variability was expected as it is a segregating population with some breeding lines expressing very high ECT levels similar to those reported for the *L. pallida* parent (Dalzell and Shelton 2002). This variability indicates that there is potential to select low ECT OC lines that are likely to have superior digestibility and forage quality. Both BC1 lines had ECT contents that were intermediate between cvv Cunningham (mean 9% DM) and Tarramba (mean 14% DM). This indicated that they should have desirable levels of ECT to promote beneficial bypass protein, but not too much ECT that can reduce palatability and protein/dry matter digestibility.

Bound condensed tannin content
Again the OC breeding lines had the highest (mean 1.5% DM) and most variable (range 0.8-2.2% DM) BCT content. Both BC1 breeding lines had higher ($P<0.05$) BCT levels than both cvv Tarramba (mean 0.5% DM) and Cunningham (mean 0.3% DM) reflecting the *L. pallida* contribution of 25% of the plant genetic make up.

Total condensed tannin content
Total condensed tannin content was calculated by adding the ECT and BCT values for each plant. As found elsewhere in a diverse range of *Leucaena* species and hybrids (Dalzell and Shelton 2002), ECT is the dominant fraction in forage accounting for >90% of TCT of the mean level of each

breeding strategy/cultivar. The absolute values measured in this study are expressed as *L. leucocephala* condensed tannin (CT) equivalents as this was the purified standard CT used to calibrate the assay. Care needs to be exercised when comparing the absolute values of the different cultivars or breeding lines both within this work and with other studies where different reference CTs were used.

4.2.4 Conclusion

The OC KX2 F_4 lines had high, although variable, condensed tannin content and lower IVDMD as has been reported elsewhere (Castillo 1993; Dalzell *et al*. 1998). However there were some individuals that were highly psyllid resistant and that had lower CT levels and acceptable digestibility that could be advanced in an OC breeding program. This indicated that high condensed tannin level is not responsible for psyllid resistance, supporting poor correlations between tannin content, digestibility and other forage quality attributes and psyllid resistance observed in other work (Castillo 1993; Purdie 1996; Mullen *et al*. 2003b). The BC1 lines derived from both KX2 F_1 and F_3 parents had *in vitro* forage quality (crude protein, IVDMD and condensed tannin content) similar to that of cvv Cunningham and Tarramba. Further backcrossing to *L. leucocephala* should further improve the forage quality of these lines by reducing condensed tannin levels and enhancing digestibility.

4.3 Entomological Program

Field adult psyllid counts
Leucaena leucocephala consistently attracted more ($P<0.05$) adult psyllids than either *L. pallida* or the F_1 KX2 hybrid, which were not significantly different. These findings were consistent with other observations of psyllid damage on these *Leucaena* species (Mullen *et al*. 2003b). Plants of all species (incl. resistant *L. pallida*) stressed by insecticide phytotoxicity (resulting from high rates of chlorpyrifos application) attracted significantly higher numbers of adults psyllids ($P<0.01$). The stressed plants may have released higher levels of volatile compounds that made them more attractive to psyllids over long distances.

Field egg and immature psyllid counts
Field collected stem tips from all *L. leucocephala* individuals had psyllid eggs (average 234±58 eggs/shoot). Over 90% of samples also had approximately 75-80 younger (instars 1-2) and older (instars 3-5) nymphs/shoot. Only 1 sample (8%) of *L. pallida* samples had 1 psyllid egg; no samples had nymphs of any age. While 33% of F_1 KX2 hybrid samples had psyllid eggs (average 17±12 eggs/shoot), few samples (8%) had older nymphs (average 10 nymphs/shoot). The egg count data mirrored the arrival rate observed in adult psyllids and is likely to be a function adult arrival rates rather than the female insects' ability to determine the suitability of the substrate for nymph development.

Assessing visual cues by reflectance tests
No significant difference in the brightness or chroma of the reflectance spectroscopy signatures for either growing points or mature leaves was observed between the 3 *Leucaena* species. It was presumed that psyllids could not detect visual differences between these species. Similarly, Lapis and Borden (1995) did not find visual cues to be important in psyllids finding susceptible *Leucaena* hosts.

Assessing olfactory cues by headspace volatile analysis
Analysis of volatile chemicals released by the 3 *Leucaena* species indicated that a terpene hydrocarbon, caryophyllene, was produced. During warm growing conditions (March, 32.3°C average maximum temperature), *L. leucocephala* and the F_1 KX2 hybrid produced significantly higher ($P<0.05$) concentrations of ß-caryophyllene than *L. pallida*. This suggested that susceptibility may be due to the production of this known insect attractant. However, under cooler conditions in April/May (27.7°C average maximum temperature) ß-caryophyllene levels in the headspace were much lower for all species and there was no difference between species. Other volatile chemicals produced by *Leucaena* species have been identified, including perillene and ß-ocimene, which may play a role in psyllid-plant interactions.

Caryophyllene field tests
Psyllids arrived at the control (no ß-caryophyllene) trap and those containing 5 different concentrations of ß-caryophyllene in equal numbers indicating that olfactory cues of caryophyllene alone was not responsible for psyllid host-plant selection.

Short range host selection
The laboratory test of adult psyllid selection of the 3 *Leucaena* species was inconclusive. Most adult psyllids contacted leaves within the 20 minute time period allowed, however there was no significant selection preference exhibited by the psyllids, irrespective of sex. This result indicated that the cues psyllids use to discriminate between host plants in the field were not effective at close range under confined laboratory conditions.

Conclusion
Given that the entomological study did not identify any definitive plant traits that conferred psyllid resistance and used to generate molecular markers for future use in the plant breeding program, the entomology research program was discontinued in 2002. Fortunately, there was no prerequisite for molecular markers to make genetic advances in psyllid resistance in the plant breeding/selection program. A robust (rapid, consistent and precise) visual assay for determination of psyllid resistance developed by the University of Hawaii (Wheeler 1988) has proved stable over a range of different environments (Mullen *et al*. 2003b,c). Furthermore, this damage rating score has been correlated to biomass yield suppression caused by psyllid attack (Mullen and Shelton 2003). This obviates the need for expensive further work to identify molecular markers. The only benefit that the identification and use of molecular markers has over the current visual assay is the potential to select for psyllid resistance in the absence of psyllid populations.

5 Success in Achieving Objectives

In October 2001, the UQ research team (and its advisors) believed it could complete 4 cycles of recurrent mass selection in 4 years followed by a year of on-farm trials and seed increase. We were optimistic about achieving this rate of progress because we had planted the first generation in Nov/Dec 2001 before the project officially started on 1 January 2002. However, as with all biological research programs, there were several reasons beyond our control, which required adjustment of our schedules. These are summarised below:

1. In June 2004 (at Milestone 3), a contract variation was sought as UQ & The Leucaena Network were unsuccessful in securing 40% of funding the NAPSWQ program of Fitzroy

Basin Association; and it became apparent that the breeding cycles were taking longer than expected so rescheduling of milestones became necessary.

2. In July 2005 (at Milestone 5), a second contract variation (V2) was sought from MLA to delay all subsequent milestones by 6 months with no change to the budget. The UQ team bore the extra cost associated with continuing the work over this 6 month period (funded from revenue generated by *Leucaena for Profit & Sustainability* training courses). The reasons for longer breeding cycles were several:
    a) Delayed flowering of desirable plants (this trait was initially selected to promote higher forage yield and minimize environmental weed potential)
    b) Poor synchronicity of flowering between pollen donors and elite selected plants prevented immediate pollinating
    c) Absence of psyllid pressure at field planting in 2005 delayed screening of populations by 4 months
    d) Prolonged period of drizzly rain in 2007 prevented hand pollination in the backcross program
    e) Bird damage at pollination and of maturing seed in years 2005 & 2007 necessitated the repeat of emasculation and pollination work.
3. The delays described above resulted in each cycle of selection taking over 18 months to complete rather than the 12 months originally envisaged. The estimate of a 12-month cycle was made based on UH experience where it was readily achieved in the Waimanalo (Oahu, Hawaii) environment.
4. In October/November 2005, a comprehensive data analysis of the F_3 generation indicated inbreeding depression for yield amongst the outcrossed hybrid progeny. In December 2005 (at Milestone 6), new breeding strategies were put in place, but no change was made to the milestone schedules even though the last milestone was now not relevant. Thus by the 30th of June 2008, whilst four cycles of breeding and selection were completed, there was no KX2 F5 seed produced and it was not possible to complete the following tasks:
    a) On-farm producer evaluations and grazing trials for cattle production of F5 plants
    b) Produce 10-20 kg of F6 seed for bulking to commercial quantities
    c) PBR preparations underway because the variety(ies) is not ready for PBR evaluation

Nevertheless, the breeding program has been outstandingly successful in achieving its central goal of producing highly vigorous, high quality psyllid-resistant leucaena breeding lines (see photos). UQ now has an array of breeding lines which will lead to the release of a new psyllid resistant cultivar.

Other objectives of the project were achieved, including the preliminary entomological study into psyllid-host plant interactions which resulted in a peer reviewed scientific publication. A number of undergraduate and postgraduate students conducted their research on aspects of the program.

The project was terminated as at the 30th of June 2008. A new commercialization phase will be initiated to complete the breeding work (see Appendix 8) and deliver a psyllid-resistant *Leucaena* hybrid to the northern Australian beef industry. However, apart from maintenance of the field site and collection of selfed seed from elite BC2 trees, no further work can occur until the commercialization contracts have been negotiated and signed.

6 Impact on Meat and Livestock Industry – now & in five years time

Whilst the new variety is not yet available to industry, its future impact will be very significant. There is heightened interest from industry in psyllid-resistant varieties, particularly in the light of heavy psyllid infestations of commercial leucaena stands during the 2007/8 growing season. These infestations are having a severe impact on the productivity of many properties in Queensland that rely on leucaena grass systems for beef production and the knowledge that a new resistant variety will be available in future gives graziers great confidence.

A number of other undergraduate and postgraduate students conducted their research on aspects of the MLA program. These young enthusiastic scientists will contribute very significantly to the beef industry in future.

Capacity building of the Leucaena Network has occurred throughout the period of funding this organization received through this project. Members of the Executive have gained experience in engaging with industry, government and research agencies. The organization is in a strong position to continue promoting the responsible use of leucaena pastures. The Network has just undertaken a review of the Code of Practice and is engaging with State Govt. agencies to ensure the Code of Practice is adopted as Queensland State Govt Policy. This process should ensure graziers in Queensland can continue to grow this valuable pasture species whilst minimizing the adverse impact of weed leucaena infestations.

The UQ/Leucaena Network partnership has also been an efficient conduit of information from industry & research/extension agencies to members. There has been an enormous amount of positive publicity for MLA arising from the project both within northern Australian and international forage agronomy/beef production forums, namely:

(a) Many articles in Queensland Country Life and other rural newspapers and magazines

(b) Excellent publicity for the program through the 23 *Leucaena for Profit and Sustainability* short courses (>460 participants) delivered to graziers between 2004 and 2008

(c) Interviews on ABC public radio and video recordings for MLA's *feedbackTV*

(d) Presentations, seminars and papers presented at industry and scientific conferences and forums held in Australia, Ireland, Paraguay and USA (Hawaii) (see Appendices 1-4).

When the commercialization process is completed, and commercial quantities of seed of the psyllid-resistant variety are available to graziers, an additional 1.2 M ha of country in coastal Queensland will be suitable for leucaena plantings. Furthermore, in the 13 M ha of subhumid Queensland suited to existing psyllid susceptible *L. leucocephala* varieties, many graziers will adopt the new variety. These opportunities will make a significant economic contribution to the northern Australian beef industry by enhancing the supply of high protein forage in existing subhumid and new coastal environments suited to leucaena development.

7 Conclusions and Recommendations

Good progress has been made in breeding psyllid resistant leucaena varieties. Several breeding lines which are vigorous, of high forage quality, and resistant to psyllids have been produced. The necessary change in breeding strategy midway through the project and the longer than anticipated generation time, has resulted in a 3-year delay in completing commercial delivery of the new variety.

A commercialization phase is recommended to complete 2 final cycles of progeny testing prior to the release of a stable psyllid-resistant backcross line. Private sector funding will be sought to purchase the right to commercialize the new variety under the protection of PBR, with additional financial support provided through the MLA Donor Company's *Partners in Innovation Program* (see Appendix 8).

Leucaena Network Executive Officer, Mr Kevin Graham, inspects one of the psyllid-resistant backcrossed lines generated by project NBP.307 in October 2008. University of Queensland agronomist and plant breeder, Dr Scott Dalzell, looks on.

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9 Appendices

Hardcopy appendices of supporting scientific papers and posters, student theses and the commercialization prospectus for the new variety that were generated by the project NBP.307 have been submitted to MLA and The Leucaena Network. The details of each document are provided below.

Scientific Papers and Posters

9.1 Appendix 1

Dalzell, S.A. (2005). *Potential for the genetic improvement of the* Leucaena *genus for forage production*. Insert from paper presented at the "Congreso Internacional de Leucaena y otras leguminosas con potencial para el Gran Chaco, Loma Plata, Paraguay, 9-11 de Marzo de 2005". 10 pages.

9.2 Appendix 2

Dalzell, S.A., Lemos de Moraes, F. and Solis Pasos, G. (2005). *Breeding a psyllid-resistant interspecific hybrid* Leucaena *for beef cattle production in northern Australia*. Poster paper presented at the XX International Grassland Congress, Dublin, Ireland.

9.3 Appendix 3

Dalzell, S.A., Lemos de Moraes, F., Solis Pasos, G. and Shelton, H.M. (2005). Breeding psyllid-resistant *Leucaena* varieties for northern Australia. Poster presented at the XX International Grassland Congress, Dublin, Ireland.

9.4 Appendix 4

Finlay-Doney, M. and Walter, G.H. (2005). Discrimination among host plants (*Leucaena* species and accessions) by the psyllid pest *Heteropsylla cubana* and implications for understanding resistance. *Agricultural and Forest Entomology*, **7**: 153-160.

Theses

9.5 Appendix 5

Lemos de Moraes, F. (2002). *Agronomic evaluation of a segregating F_2 population of KX2 (*L. pallida *x* L. leucocephala *ssp.* glabrata*) hybrid* Leucaena. Master of Agricultural Studies, The University of Queensland. 48 pages.

9.6 Appendix 6

Solis-Pasos, G. (2005). *Breeding psyllid resistant* Leucaena *varieties using interspecific hybrids between* Leucaena leucocephala *(Lam.) de Wit ssp.* glabrata *(Rose) S. Zarate and* L. pallida *(Britton & Rose)*. Master of Philosophy, The University of Queensland. 108 pages.

9.7 Appendix 7

Litzow, E.J. (2007). In vitro *study of the forage quality of the potential psyllid-resistant* Leucaena *hybrid.* Fourth Year Project, The University of Queensland. 49 pages.

General

9.8 Appendix 8

Dalzell, S.A. and Shelton, H.M. (2008). *Prospectus for the commercialisation of the new psyllid-resistant* Leucaena *variety*. 33 pages.